THE EFFECTS OF THERMOPERIOD ON THE CARBON DIOXIDE UPTAKE AND COMPENSATION POINT OF THE PINEAPPLE PLANT, *ANANAS COMOSUS* (L.) MERR.

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ABSTRACT

THE EFFECTS OF THERMOPERIOD ON THE CARBON DIOXIDE UPTAKE AND COMPENSATION POINT OF THE PINEAPPLE PLANT, ANANAS COMOSUS (L.) MERR.

The effects of several thermoperiods on the CO_2 compensation points and CO_2 uptake rates of the youngest fully expanded leaf of pineapple (Ananas comosus (L.) Merr.) were measured. Three to nine days of adaptation at a specific thermoperiod were required to obtain uniform uptake rates.

CO_2 compensation points, determined just after the lights were turned on, varied from a high of 170 ppm at a 35 C light-15 C dark thermoperiod to 0 ppm at a constant temperature of 25 C. Values for the other thermoperiods generally decreased as the difference between the light and dark temperature was decreased. The results indicate involvement of different enzyme systems in CO_2 fixation at the different thermoperiods. The CO_2 equilibrium, with no CO_2 supplied, was monitored continuously for periods up to 4 days. At 20 C in the dark essentially all the CO_2 was extracted from the sealed chamber. Extraction efficiency of the leaf in the dark decreased with increasing temperature. In the light at a 35 C light-30 C dark thermoperiod, the CO_2 concentration ranged from 69 to 200 ppm. Lower maxima were measured at a dark temperature of 20 C and at a 25 C light-20 C dark thermoperiod. No diurnal cycling in the CO_2 concentration was observed in continuous light or dark at constant temperatures of 20 or 25 C.

When the CO_2 concentration was maintained at 300 ppm, CO_2 uptake was maximal at the constant thermoperiods and ranged from 53 to 109 mg dm^{-2} for 24 hours as temperature was increased from 15 to 30 C.

Fixation decreased to 50 percent of the maximum at a constant temperature of 35 C. At a constant dark temperature, the mg of CO_2 fixed in the light and total mg for 24 hours decreased as the temperature of the light period was increased from 15 to 40 C. When the difference between the light and dark temperatures was 5 C or greater, 15 to 20 mg CO_2 dm^{-2} were fixed in the dark even at temperatures of 30 C. The percent of CO_2 fixed in the dark ranged from 0 at a constant temperature of 25 C to 86 at a 40 C light-25 C dark thermoperiod. Total CO_2 fixation decreased as the percent of CO_2 fixed in the dark increased. The dominant factor determining CO_2 fixation rates of pineapple under these conditions appeared to be the amount of CO_2 fixed in the dark. Dark fixation was determined primarily by the difference between the light and dark temperature and, to a lesser degree, by the actual temperature.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

INTRODUCTION

Pineapple growing regions of the world extend from 30° north latitude through the equator to 30° south latitude (Collins, 1960). Large differences in plant type have been observed in the pineapple growing regions of Africa, which extend from 12° north to 30° south latitude (Dr. J. B. Smith, personal communication).[1] These variations include changes in the width, curvature and amount of water storage tissue of the leaves. Dr. Smith had attributed these variations to genotype, but has recently attributed them primarily to thermoperiod and absolute temperature differences which occur with changes in latitude. Similar, though less marked variations in plant type have been reported to occur in Hawaii at different elevations.

Variations in the growth rate and development of pineapple at different latitudes have also been reported. Dodson (1966) reported that five years were required for the production of a first or plant crop and ratoon or second crop in Swaziland (27° south latitude). Dr. W. G. Sanford[2] (personal communication) reported that approximately 15 months were required to produce a plant crop in Ivory Coast (5° - 12° north latitude). The ratoon crop was assumed to be poor because the plants had insufficant carbohydrate reserves to initiate sucker development (shoots developing from axillary buds near the base of the plant). In Hawaii (20° north latitude) plant crop fruit are harvested

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[1]Dr. J. B. Smith, Former director, Pineapple Research Institute of Hawaii.

[2]Dr. W. G. Sanford, Department of Agronomy and Soil Science, University of Hawaii, Honolulu, Hawaii.

after 20 to 22 months and at least one ratoon crop is harvested approximately one year after the plant crop.

Pineapple is grown in Hawaii from elevations near sea level to about 2,000 feet above sea level. Pineapple leaves tend to become narrower, are more rigid with a sharper degree of curvature and, according to some field workers, have thicker water storage tissue on the adaxial side of the leaf when grown at the higher elevations. These differences appear to be attributable primarily to temperature rather than to sunlight or soil moisture levels.

Data on stomatal opening of succulents and xerophytes is sparse and, in some cases, contradictory. Most of the research results indicate that the stomata of xerophytes open in the dark and massive dark CO_2 fixation occurs at this time. Yoder (1969) reported that both the thermoperiod and absolute temperature had a considerable effect on transpiration rates of pineapple. Transpiration and apparent stomatal opening (leaf resistance) during the day and night were determined more by the day-night temperature differential than by absolute temperature.

In the "grand period of growth" a pineapple plant approximately doubles its weight every three months. It is difficult to account for the relatively high dry matter accumulation rates observed for pineapple by the low rates of dark CO_2 uptake reported in the literature. Yoder (1969) found the highest rates of transpiration occurred during the day with a constant thermoperiod of 30^{o} C indicating that the stomata were open in the light. It is likely that CO_2 uptake occurred in the light during these periods.

These variations in growth rate and plant type which occur with changing altitude and latitude and variations in transpiration rate and apparent stomatal opening which occur with thermoperiod indicate a considerable effect of climate.

The objectives of this research were to evaluate the effects of various thermoperiods on the CO_2 exchange rates and CO_2 compensation points of a single attached pineapple leaf.

REVIEW OF LITERATURE

Carbon Dioxide Compensation Points

The carbon dioxide compensation point is the equilibrium CO_2 level reached when no CO_2 is added to a well illuminated plant or leaf in a closed chamber. The CO_2 compensation point has been reported to be relatively constant within a given species, but varied greatly between species under the same environmental conditions (Downton and Tregunna, 1968; Moss, et al., 1969; Moss, 1962).

The carbon dioxide compensation point can be used to indicate whether CO_2 is fixed predominantly by carboxydismutase (C-3 plants) or phosophenolpryuvate (PEP) carboxylase (C-4 plants). Those species which have compensation points of 10 ppm CO_2 or less have large amounts of PEP carboxylase and fix carbon via the C-4 pathway. Photosynthetic rates of C-4 plants are approximately twice those of species having compensation points of 50 ppm CO_2 or greater (Downton and Tregunna, 1968; Hatch, et al., 1967; Moss, 1962). Oxygen concentration did not affect the photosynthetic rate of C-4 plants, but low oxygen concentrations greatly enhanced rates of CO_2 uptake by C-3 plants (Bjorkman, 1968; Downs and Hesketh, 1968; Hesketh, 1967). Plants which had a high CO_2 compensation point in 21 percent oxygen, had compensation points of 5 ppm CO_2 or less in one to two percent oxygen (Tregunna and Downton, 1967; Tregunna and Krotkov, 1966).

Light is known to affect the carbon dioxide compensation point at low intensities, however, it has little affect at high intensities. The specific intensities were reported to vary among species (Heath, 1969; Moss, 1962). Temperature has a greater affect on the carbon dioxide

compensation point than does light. The CO_2 compensation point is lowest at the optimum temperature for the specific species and increases as the temperature is increased or decreased from the optimum. The optimum temperature has been reported to be the temperature where the enzymatic systems have their greatest affinity for CO_2 (Heath, 1969; Downton and Tregunna, 1968; Heath and Orchard, 1957).

The work of Moss (1962) and Downton and Tregunna (1968) indicated a steady state CO_2 compensation point. Jones and Mansfield (1970) reported a circadian rhythm in coffee and a Bryophyllum species under continuous light. They pointed out that the maximum values of the compensation points varied considerably, but the lower limits were fairly constant.

The equilibrium CO_2 concentration obtained in the dark for plants having crassulacean acid metabolism (CAM) is not a CO_2 compensation point in the strict sense of the definition given previously. Although little or no data are available, the effects of temperature on dark fixation of CO_2 would indicate a temperature effect on the equilibrium CO_2 concentration could be expected.

Under suitable conditions plants which have CAM can remove nearly all the CO_2 from a dark enclosed environment (Thomas and Ranson, 1954). The rate and maximum acid levels in the dark were reported to be temperature dependent (Ranson and Thomas, 1960). Bennet-Clark (1933) reported that temperatures of 20 C or less favored dark acidification, whereas 30 C or higher enhanced dark deacidification. Ranson (1954) reported that acid accumulation decreased as temperatures were raised from 12 C to 30 C.

Carbon Dioxide Exchange Rates

In CAM type plants the malic-citric acid concentration fluctuates diurnally. Such a diurnal cycle has been reported for pineapple (Sideris, et al., 1948). The rate of depletion of stored acid in the light was dependent on light intensity and temperature (Ranson and Thomas, 1960). Also, the quantity of malic acid synthesized during dark acidification was reported to be dependent upon the light intensity and amount of photosynthate produced in the preceeding dark period (Ranson and Thomas, 1960; Sideris, et al., 1948). High levels of acid were correlated with high light intensities (Sideris, et al., 1948), but as with Jones and Mansfield (1970) the lower limits were fairly constant (H. Y. Young).[1]

The work of Sideris, et al. (1948) was confirmed by Seshagiri and Suryanarayanamurthy (1957). In addition, they reported a cycling of the acid level when the pineapple plant was subjected to long periods of darkness.

The first CO_2 uptake data over a 24 hour period for a whole pineapple plant were reported by Joshi, et al. (1965). Their work showed a maximum CO_2 uptake rate during the dark period with CO_2 evolution occurring from morning until midday. Theoretical photosynthetic rates were approximately 0.60 mg CO_2 dm^{-2} hr^{-1} over an 11 hour photoperiod. Transpiration ratios (g H_2O lost / g dry matter) were approximately 50. It should be noted that the plants were not preconditioned to the chamber and only two 24 hour experiments were run at different times.

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[1]Young, H. Y., Personal communication with the author.

Whereas Joshi, et al. (1956) reported the greatest percentage of CO_2 uptake occurred at night, Neales, et al. (1968), using a single attached pineapple leaf, found that only 37 percent of the total CO_2 uptake by pineapple occurred in the dark. Neales, et al. (1968) reported uptake decreased for the first part of the day, but no net loss of CO_2 was observed. Maximum exchange rates were 0.56 mg CO_2 dm^{-2} hr^{-1} for the day and 0.41 mg CO_2 dm^{-2} hr^{-1} for the night. Transpiration ratios were 64 for the day, 32 for the night and 53 for the 24 hour period.

Radioactive carbon labeling studies have shown that the pineapple plant fixes carbon in the light as well as in the night. A study by Gardner[2] showed that after labeling with $^{14}CO_2$ for ten minutes in the dark, 84 percent of the radioactivity was accounted for in malate. The ratio of ^{14}C was 3:1 on the C-4 and C-1 carbon atoms of malate, as was reported for other CAM plants (Ranson and Thomas, 1960). Labeling for four seconds in the dark gave similar results, but with less total uptake. A ten minute exposure to $^{14}CO_2$ in the light resulted in incorporation of 43.8 percent of the ^{14}C into malate and 43.4 percent in sugars or sugar phosphates. Labeling of malate was approximately the same in the light as in the dark except recycling of intermediates in the light had resulted in the labeling of carbons 2 and 3.

As was reported for other CAM plants by Thomas and Ranson (1954) and Bennet-Clark (1933) pineapple plants were found to accumulate the highest levels of acid during cool nights and warm days (Seshagiri and

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[2]Gardner, H. W. In the files of the Pineapple Research Institute of Hawaii.

Suryanarayanamurthy, 1957). Kent[3] found the optimum temperature for maximum dark CO_2 fixation by excised leaves of pineapple was 17 C. Above 31 C there was a net loss of CO_2.

It was generally believed that pineapple plants transpired during the night and that the stomata close during the day preventing water loss. One study (Kent[4]) of stomatal movement showed that pineapple stomata begin closing shortly after daybreak (average closing time of 20 minutes) and stayed closed until midafternoon. The stomata remained fully open throughout the night. Yoder (1969) studied the transpiration rates of small pineapple plants and found the highest rates of transpiration occurred at 30 C during the day. In only a few experiments did night transpiration rates exceed rates measured during the day.

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[3]Kent, M. J. In the files of the Pineapple Research Institute of Hawaii.

[4]Ibid.

METHODS AND MATERIALS

Pineapple plants, Ananas comosus (L.) Merr., variety Smooth Cayenne, were grown in aerated solution and sand culture for approximately 12 to 14 months. One liter of nutrient solution (Appendix A, Table I) was supplied twice a week to the sand cultured plants. The sand was flushed approximately once every two weeks with tap water to prevent salt accumulation. Solution cultured plants were grown in 11 liter glazed crocks. The solution was changed once or twice a month. The longest leaves on the plant ranged up to 70 cm.

A large plexiglas growth chamber (Appendix A, Figure 1), leaf chamber (Appendix A, Figure 2) and CO_2 injection and control system (Appendix A, Figure 3) were developed to provide a wide range of environmental conditions over an extended period of time. A whole pineapple plant was placed in the large chamber. CO_2 data were collected from a single 'D' leaf (Krauss, 1948) isolated in the leaf chamber.

Temperature control was achieved in the large growth chamber (approximately $1m^3$) with a nichrome wire heater and a vertical fin heat exchanger through which cool water was passed. A thermostatically controlled 3/4 ton refrigeration unit was used to cool the water supply for the heat exchanger. By adjusting the water supply temperature and controlling the heater circuit with a rheostat the temperature of the chamber could be maintained within ± 1 C over the range from 15 C to 40 C. A 24 hour timeclock was wired into the heater circuit to turn the heater on or off to establish the desired thermoperiod. Where the day and night temperatures differed, the duration of each was 12 hours.

Light was supplied by four 500 watt incandescent flood lamps suspended in ten cm of cool running water to reduce the infrared radiation emitted by the lamps. The average light intensity along the leaf under test was 7600 micro watts cm^{-2} (red plus blue) as measured with an IL 150 plant growth photometer. The value was approximately equal to 1400 foot candles as determined by a calibration curve between a Weston foot candle meter and the IL 150 plant growth photometer. Both the plant and isolated leaf received approximately the same radiation in the same horizontal plane. The photoperiod, beginning at 0700 hours was maintained at 12 hours and was controlled by a 24 hour timeclock.

All CO_2 data were collected using a small temperature controlled chamber containing a single 'D' leaf. Thermocouples were used to gather leaf temperature data from the isolated leaf and other leaves in the same horizontal plane on the entire plant. This data was used to maintain the isolated leaf at the same temperature as the plant. A small heat exchanger was fitted inside the leaf chamber and the air was circulated throughout the chamber and heat exchanger by a small fan and air duct. The leaf chamber had a water jacket on one side to provide an additional cooling surface. A small refrigeration unit, thermostat and 100 watt heater were used to control the temperature of the water supply to the heat exchanger and water jacket of the chamber. Without an independent cooling system the temperature of the leaf chamber was 3 C to 5 C above the plant chamber due to the greenhouse effect. The leaf rested on a cotton thread support to allow complete air circulation around the leaf.

The CO_2 injection and control system was similar to that of Hoffman, et al. (1969) and consisted of a master double set point relay,

automatic reset timers, counters, delay relay, pumps and solenoids. The output to the ammeter of the Beckman 215 infrared gas analyzer (IRGA) was shunted to the double set point relay. The system was capable of maintaining the CO_2 concentration between 0 and 600 ppm with as little as a 10 ppm differential. A schematic flow chart is given in Figure 1.

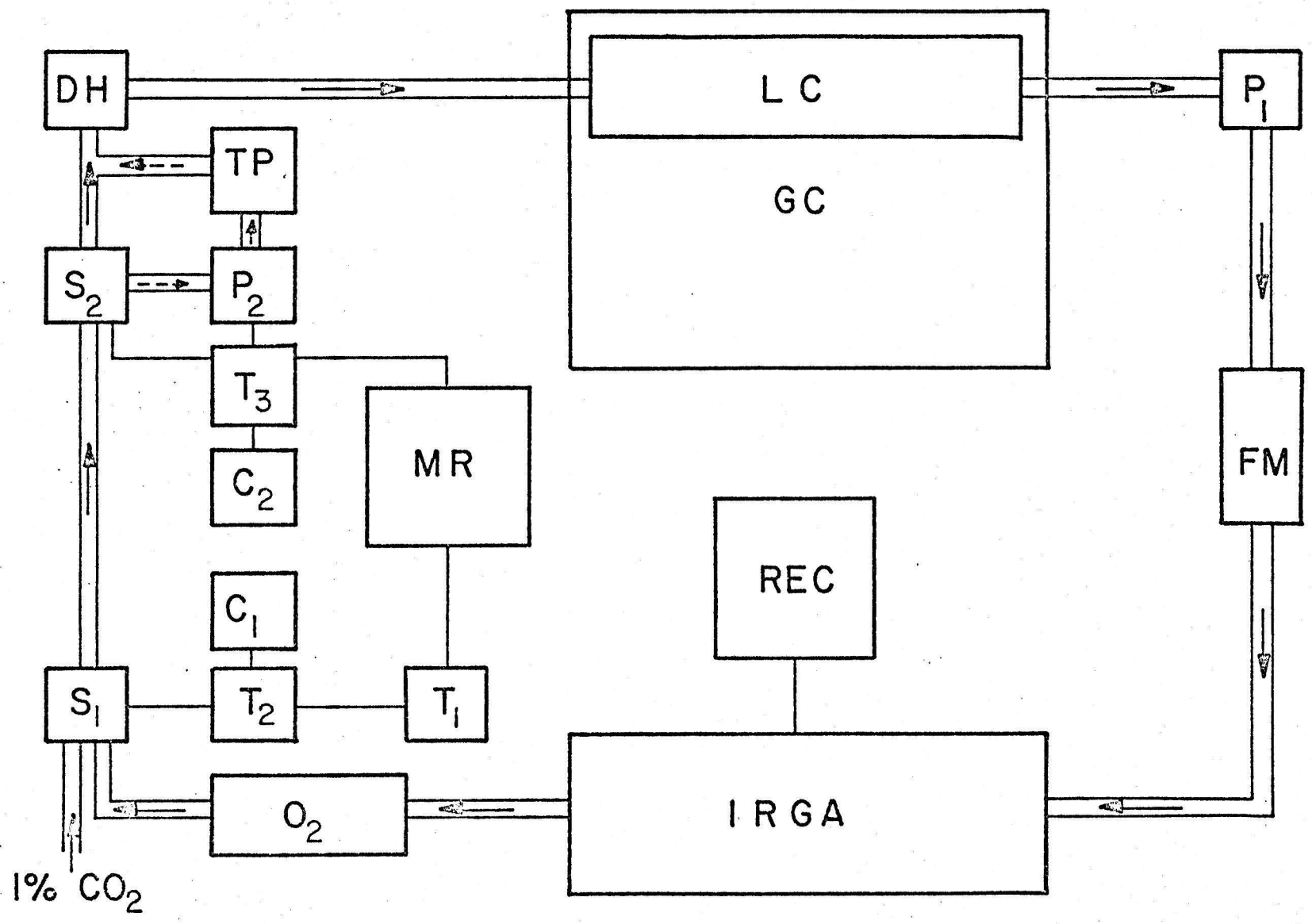
The CO_2 was continuously monitored by recirculating the air within the closed system of the leaf chamber, IRGA, and oxygen analyzer at 1.75 liters per minute. The volume of the leaf chamber and instrumentation system was approximately 6.5 liters. The time lag from CO_2 injection to measurement of a concentration change was just over 15 seconds. To eliminate the formation of condensate on the tubing walls or in the IRGA sample cell, the sampled air was passed through a flask chilled below the dewpoint by submerging it in the large cooling tank.

Methods

All data were collected on a single 'D' leaf by enclosing the leaf in the leaf chamber previously described. One end plate was removable and fitted with a rubber gasket. The removable end plate of the leaf chamber and rubber gasket were fitted onto the leaf and sealed on both sides with melted paraffin. The leaf was then rested on the thread support in the chamber and the end plate secured to the chamber with bolts. To check for air leaks in the chamber and wax seals around the leaf, the leaf chamber CO_2 was depleted using a soda-lime CO_2 trap. CO_2 from a cyclinder was then blown around the chamber. Even minute leaks resulted in large fluctuations of the IRGA output due to the large CO_2 concentration differential between the chamber (near 0 ppm CO_2) and the cylinder gas (near 100 percent CO_2).

Figure 1. Flow chart of the closed circuit CO_2 control and analysis system.

LC	Leaf Chamber
GC	Growth Chamber
P_1	Pump To Circulate Sampled Air Through System
FM	Flow Meter
IRGA	Infrared Gas Analyzer
Rec	Recorder For IRGA Output
O_2	Oxygen Analyzer
S_1	Control Solenoid For CO_2 Injection
T_1	30 Second Delay Relay
T_2	CO_2 Injection Timer (0 - 15 seconds)
C_1	Counter For CO_2 Injection Circuit
S_2	Solenoid To Change Sampled Air Through CO_2 Trap
P_2	Pump To Force Air Through CO_2 Removal Pathway
T_3	Timer For CO_2 Removal (0 - 60 seconds)
TP	Soda-Lime CO_2 Trap
DH	Dehydrator For Sampled Air


DH
LC
GC
P1
TP
S2
P2
T3
MR
C2
FM
REC
C1
S1
T2
T1
O2
IRGA
1% CO2

Measurements were made of the CO_2 compensation point and of changes in the long term equilibrium between CO_2 intake and CO_2 evolution. The CO_2 compensation points were determined according to Moss (1962). The compensation point is defined as the equilibrium CO_2 concentration reached when no CO_2 is added to an illuminated plant or leaf in a closed chamber. The continuous equilibrium between CO_2 intake and CO_2 evolution (E CO_{2i}/CO_{2e}) was determined in a closed system to which no external CO_2 was supplied. This equilibrium is dependent on the rates of dark and light fixation and respiration. The E CO_{2i}/CO_{2e} was determined in a manner similar to the compensation point. Depending on the experiment, the leaf was or was not illuminated and the period over which it was evaluated extended from several hours to several days.

The initial CO_2 concentration for the compensation point and E CO_{2i}/CO_{2e} studies was 300 ppm. The compensation points were determined just after the lights were turned on (0700 hours). The CO_2 injection circuit was automatically disconnected from the system when the lights came on and the leaf was allowed to deplete the CO_2 supply in the closed system. The compensation point was taken as the minumum stable CO_2 concentration; i.e. no change in the concentration over a period of several minutes. Prior to determining the CO_2 compensation point, the plant was adapted to the specific thermoperiod. The plant was assumed to be adapted when the CO_2 uptake rates were similar for two consecutive days. Usually three days were required to achieve adaptation to a given thermoperiod.

Data on E CO_{2i}/CO_{2e} represent the change in the CO_2 concentration due to diurnal cycling over extended periods of time. Whereas the

compensation points were always determined at 0700 hours in the light, E CO_{2i}/CO_{2e} values include both illuminated and nonilluminated conditions.

CO_2 uptake rates were determined after the plant became adapted to the specific environmental conditions of the chamber. The CO_2 uptake rates were determined by maintaining the CO_2 concentration between 300 and 350 ppm and monitoring the amount of CO_2 injected into the system. The amount of CO_2 injected into the system was determined by counting the number of pulses (automatically recorded) and multiplying by the mg of CO_2 per pulse.

The average amount (mg) of CO_2 injected into the system at each pulse was determined by injecting 50 one second pulses of gas (one percent CO_2 and 99 percent N_2) through the system at a pressure of three pounds and trapping the CO_2 in a soda-lime trap. After each 50 pulses the traps were weighed to determine the mg of CO_2 per pulse.

When the chamber CO_2 concentration exceeded the upper limit set on the master meter relay (usually 350 ppm) it activated the high CO_2 removal circuit. This circuit activated the removal solenoid, counter and pump to reroute the air flow through a soda-lime trap. Each activation of the removal solenoid was automatically counted. The total mg CO_2 removed was determined by multiplying the number of counts by the average mg of CO_2 removed per activation.

The average mg of CO_2 per count removed from the system was determined by manually operating the CO_2 injection circuit, increasing the chamber CO_2 concentration above the high set point which automatically activated the removal circuit. The mg of CO_2 removed for each operation of the removal circuit were determined gravimetrically.

A standard curve was drawn to convert the standard curve of the IRGA (ppm) to mg CO_2. By removing all CO_2 from the chamber and manually activating the injection circuit it was possible to convert ppm to mg for any increment of the IRGA standard curve (0 to 600 ppm). Thus the recorder output could be used as a check on the automatic counting circuits.

Since the system was closed and only a CO_2, N_2 gas mixture was injected into the system the O_2 concentration gradually fell below 20 percent due to respiration and dilution. Also in some cases pressure built up within the system due to the high rates of CO_2 injection (99 percent N_2). To maintain the pressure and O_2 concentration near atmospheric levels the system was opened as needed just prior to 0700 hours.

The experiments were designed to evaluate the effect of thermoperiods having diurnal ranges from 0 to 20 C and temperatures ranging from 15 to 40 C on CO_2 compensation points and uptake rates of a single attached pineapple leaf. Four different plants grown in both sand and solution culture were used. The following experiments were performed. The photoperiod was 12 hours unless otherwise noted.

1. 30 - 20 C day-night thermoperiod. The CO_2 equilibrium was recorded continuously over a ten day period. No external CO_2 was supplied.
2. 35 - 30 C day-night thermoperiod. The CO_2 equilibrium was recorded continuously over a seven day period. No external CO_2 was supplied.
3. 20 C day-night thermoperiod. Continuous dark. The CO_2

equilibrium was recorded continuously over a seven day period. No external CO_2 was supplied. The plant used in experiments 1 through 3 was grown in sand.

4. 25 - 20 C day-night and 30 - 20 day-night thermoperiods. Three days at each thermoperiod to study the effects of rapid change from one thermoperiod to another and adaptation to the new thermoperiod. The plant used was grown in sand.
5. 15 C night with 20, 25, 30 and 35 C day thermoperiod. 20 C night with 25, 30 and 35 C day thermoperiods. Hourly uptake rates and compensation points were determined. The plant used for the above series was grown in sand culture and water stressed.
6. 25 C night with 20, 25, 30, 35 and 40 C day thermoperiods.
   30 C night with 30, 35 and 40 C day thermoperiods.
   35 C day-night thermoperiod.
   Hourly uptake rates and compensation points were determined.
   The above tests were made using two plants grown in sand culture.
7. 35 C day with 15, 20, 25, 30 and 35 C night thermoperiods. Hourly uptake rates and compensation points were determined with a plant grown in sand culture.
8. 15 C night with 15, 20, 25, 30 and 35 C day thermoperiods.
   20 C night with 20, 25, 30 and 35 C day thermoperiod.
   Hourly uptake rates and compensation points were determined with a plant grown in solution culture.
9. 25 C day-night thermoperiod. Continuous light for eight days. Hourly uptake rates were determined. The plant was grown in nutrient solution.

10. 20 C day-night thermoperiod. Continuous dark for three days. Hourly uptake rates were determined. The plant was grown in nutrient solution.

RESULTS

Adaptation to Chamber

The environmental change occurring when a plant was first transferred from the greenhouse to the chamber or when thermoperiods were changed resulted in erratic CO_2 uptake patterns for from one to nine days. After this adjustment period a uniform rhythm followed.

When a plant was transferred to the chamber it evolved CO_2 during the first day. Some plants evolved enough CO_2 to raise the chamber concentration above 600 ppm CO_2, the maximum limit of the detection circuit. Under such a situation CO_2 was automatically removed from the closed system to maintain a concentration near 300 ppm.

In most cases the CO_2 uptake cycle that was established by the third day remained constant until the chamber conditions were changed. However, when changing from a thermoperiod where the difference between the day and the night temperature was 0 ($\Delta T = 0$, 35 C day-35 C night) to a thermoperiod where ΔT was 15 C, (35 C day-20 C night) as many as nine days were required to establish a uniform cycle. Thermoperiod changes that resulted in increased CO_2 uptake rates did not require a long adaptation period. The longer than normal adaptation period was only noticed when uptake rates had increased to a maximum and the next thermoperiod change resulted in a decreased uptake rate.

Once the plant became adapted to a specific environment, in the light a change in temperature of 1 to 2 C for as little as five minutes resulted in an immediate change in uptake rates. The uptake rate increased if the temperature change decreased ΔT and decreased if the temperature change increased ΔT.

Equilibration Studies - No External CO_2 Supplied

The studies of the continuous equilibrium between CO_2 intake and CO_2 evolution (E CO_{2i}/CO_{2e}) were run to determine if the CO_2 compensation point was a steady state point after the plant had become adapted to the chamber or if diurnal cycling continued to occur. The importance of proper adaptation is shown in the results of Figure 2. A uniform diurnal rhythm was not obtained until after four days of adaptation to the environment.

A thermoperiod effect on E CO_{2i}/CO_{2e} was noted while maintaining the plant without an external CO_2 supply (Table I). At a night temperature of 20 C, dark CO_2 fixation by the leaf lowered the CO_2 to a concentration near 0 ppm at all thermoperiods examined. Increasing the night temperature to 30 C resulted in an increase in the night CO_2 level to 20 ppm at a 30 C day and 40 ppm at a 35 C day. The minimum E CO_{2i}/CO_{2e} levels were always reached during the night, but not always at the same time. The time at which the minimum values were reached depended upon the specific thermoperiod and varied from as early as 2300 hours to as late as 0700 hours (Figure 3 A, B and C). When the minimum values were reached they were maintained throughout the balance of the night. During the day the E CO_{2i}/CO_{2e} values responded to increased temperatures as well as to increased ΔT. The higher the day temperature and the greater the value of ΔT, the higher was the maximum E CO_{2i}/CO_{2e} value. The time at which the maximum equilibrium value was reached was also affected by temperature.

The effects of a prolonged dark period on the E CO_{2i}/CO_{2e} of a plant that had been adapted to a 12 hour photoperiod and a 20 C day-night thermoperiod are shown in Figure 4 A and B. The leaf took up CO_2 during

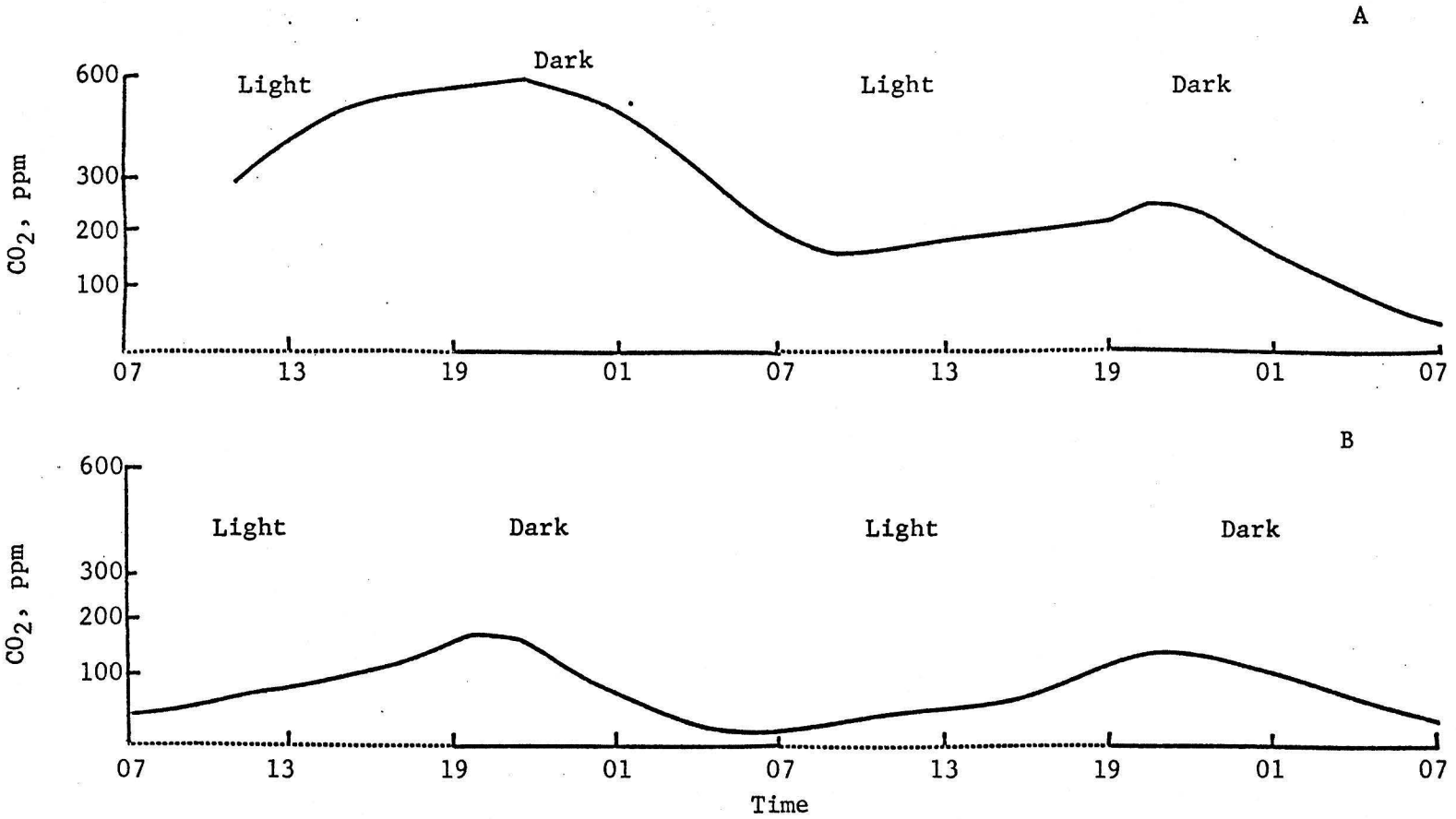
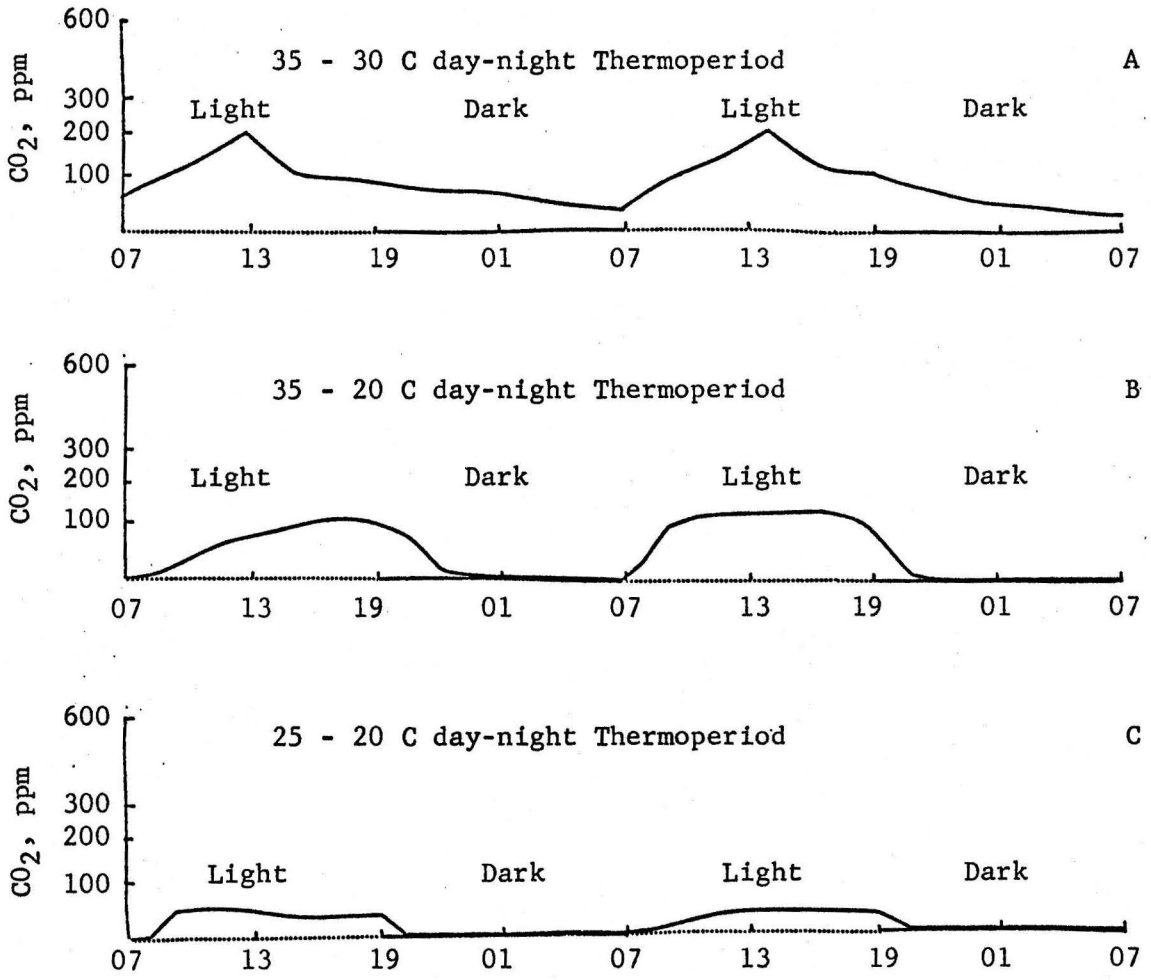


FIGURE 2. THE CONTINUOUS EQUILIBRIUM BETWEEN CO_2 INTAKE AND CO_2 EVOLUTION OBTAINED DURING THE FIRST FOUR DAYS AN ATTACHED "D" LEAF WAS IN A CLOSED CHAMBER WITH NO EXTERNAL CO_2 SUPPLIED AND AT A TEMPERATURE OF 20 C.

FIGURE 3. THERMOPERIOD EFFECTS ON THE CONTINUOUS EQUILIBRIUM BETWEEN CO_2 INTAKE AND CO_2 EVOLUTION OF AN ATTACHED 'D' LEAF IN A CLOSED CHAMBER WITH NO CO_2 SUPPLIED.


600
300
200
100
CO_2, ppm
35 - 30 C day-night Thermoperiod
A
Light
Dark
Light
Dark
07
13
19
01
07
13
19
01
07
35 - 20 C day-night Thermoperiod
B
25 - 20 C day-night Thermoperiod
C
HOURS

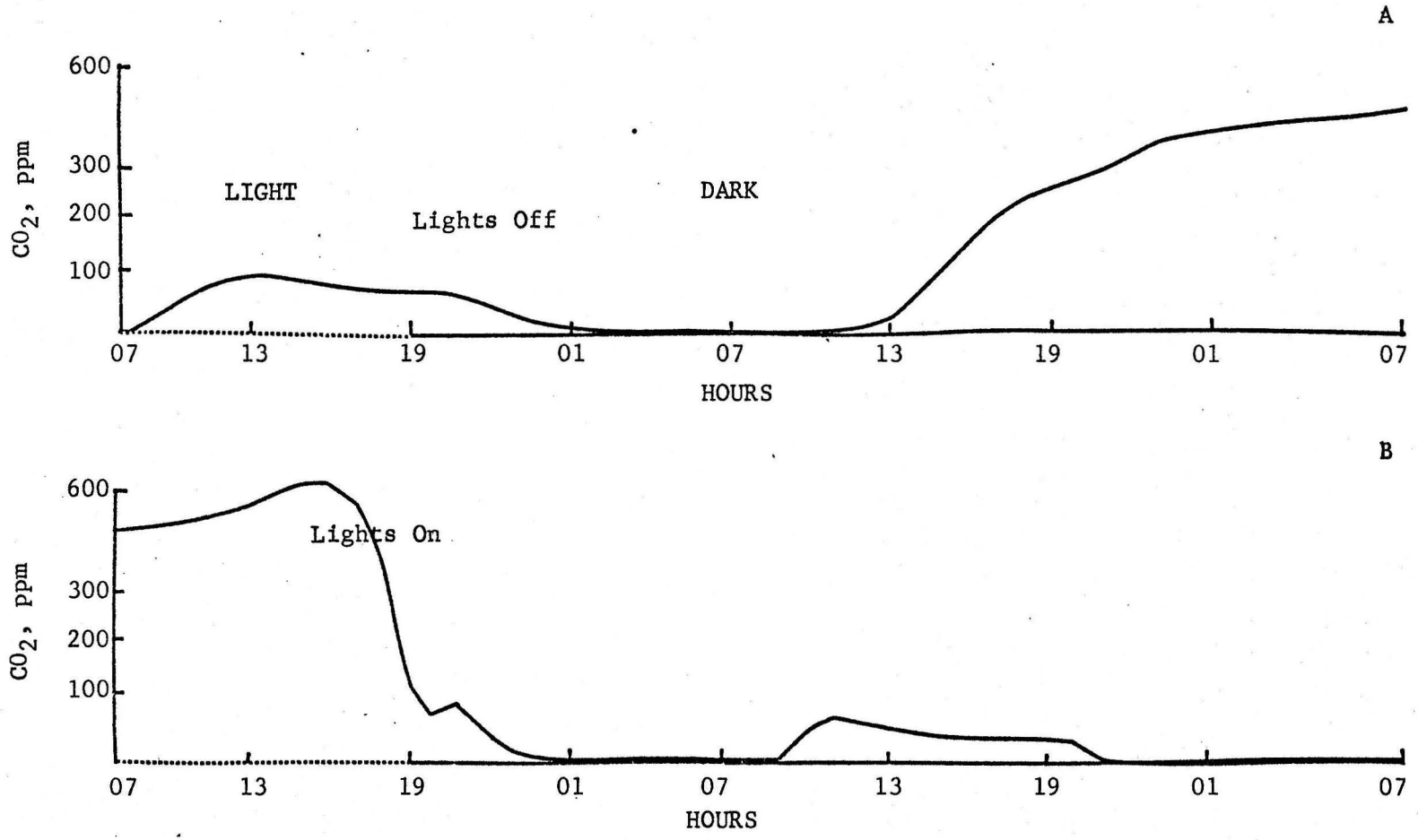


FIGURE 4. THE EQUILIBRIUM BETWEEN CO_2 INTAKE AND CO_2 EVOLUTION OBTAINED FROM A SINGLE ATTACHED PINEAPPLE LEAF SUBJECTED TO PROLONGED PERIODS OF CONTINUAL DARKNESS AT 20 C.

the first 6 hours of the dark period reducing the concentration to near 0 ppm. The CO_2 concentration was maintained at 0 ppm for about the next 12 hours. Evolution of CO_2 then occurred for the next 25 to 30 hours. When the chamber CO_2 concentration reached the maximum limit of the detection circuit the lights were turned on and CO_2 uptake began immediately (Figure 4 B).

TABLE I

MAXIMUM DAY AND MINIMUM NIGHT CO_2 EQUILIBRIUM CONCENTRATIONS OBSERVED AT NIGHT TEMPERATURES OF 20 AND 30 C AND DAY TEMPERATURES OF 20, 25, 30 AND 35 C

Day Temperature C	Night Temperature, C 20		30	
	CO_2 Conc., ppm Day	Night	CO_2 Conc., ppm Day	Night
20	40	0	a	a
25	60	0	a	a
30	140	0	170	20
35	510	0	200	40

a - No experiments were run at these thermoperiods.

CO_2 Compensation Points

The CO_2 compensation points for a specific thermoperiod were determined after an adaptation period (usually three days) with the chamber CO_2 concentration maintained between 300 to 350 ppm. In most instances different thermoperiods were evaluated by keeping the night temperature constant while varying the day temperature. The

determination was made in the first one to two hours after the lights were turned on (usually 0700).

In general the compensation points increased as day temperatures were increased while the night temperature was held constant. With the exception of the 30 - 30 C thermoperiod the compensation point decreased as the night temperature was increased for a given day temperature (Table II). Reduction of the chamber CO_2 concentration to zero (by absorption of the CO_2 with soda-lime) after a compensation point had been established and allowing the plant to re-equilibrate, the CO_2 concentration increased to a level equal to that of the original compensation point. If too much time elapsed between the two determinations the values did not coincide, having been affected by diurnal cycling.

TABLE II

THE EFFECT OF THERMOPERIOD ON THE CO_2 COMPENSATION POINT OF A SINGLE PINEAPPLE LEAF JUST AFTER LIGHTS ON

Night Temperature C	Day Temperatures, C 15	20	25	30	35	40
15	40	50	60	95	170	a
20	a	40	60	75	a	a
25	a	0	0	25	125	160
30	a	a	a	30	45	75
35	a	a	a	a	40	a

a - No experiments were run at these thermoperiods.

Minimum compensation points were reached at the constant thermoperiods. At a given night temperature the compensation point

increased as the day temperature and ΔT increased. For the one case with a 20 day - 25 night thermoperiod (ΔT = -5C) the compensation point was zero.

The length of time required to reach a compensation point was also affected by thermoperiod (Table III). In most instances the time required was greatest at the low compensation points. A calculation of the rate of decline (ppm CO_2/minute) in the CO_2 concentration (Table IV) showed some inconsistencies at day temperatures of 20 and 30 C. However, for any given night temperature the rate increased as the day temperature and the compensation point increased. Generally the rate of CO_2 depletion decreased as night temperature was increased.

TABLE III

THE EFFECT OF THERMOPERIOD ON THE TIME REQUIRED TO REACH THE CO_2 COMPENSATION POINT

Night Temperature C	Day Temperature, C 15	20	25	30	35	40
15	100	65	55	50	20	a
20	a	100	60	50	a	a
25	a	95	80	60	30	25
30	a	a	a	65	60	50
35	a	a	a	a	70	a

a - No experiments were run at these thermoperiods.

The compensation point was also affected by short term temperature variation. After the compensation point had been reached and CO_2 evolution increased to the point where the chamber CO_2 concentration

TABLE IV

THE EFFECT OF THERMOPERIOD ON THE AVERAGE RATE OF CO_2 DEPLETION (PPM/MINUTE) FROM THE LEAF CHAMBER SYSTEM IN DETERMINING PINEAPPLE CO_2 COMPENSATION POINTS

Night Temperature C	Day Temperatures, C					
	15	20	25	30	35	40
	CO_2 Depletion Rate, ppm/minute					
15	2.60	3.85	4.36	4.10	6.50	a
20	a	2.60	4.00	4.50	a	a
25	a	3.16	3.75	4.58	5.83	5.60
30	a	a	a	4.15	4.25	4.50
35	a	a	a	a	3.71	a

a - No experiments were run at these thermoperiods.

began to rise, a change in temperature resulted in a 'new' compensation point. In the specific case of a 35 C day - 25 C night thermoperiod a compensation point of 125 ppm CO_2 was obtained. The day temperature was then reduced from 35 to 30 C. The plant responded immediately with CO_2 uptake exceeding CO_2 evolution and the chamber CO_2 concentration was reduced to 75 ppm. Increasing the temperature to 35 C again caused a shift in the balance between uptake and evolution resulting in an increase in the chamber concentration. The chamber CO_2 concentration increased above the original compensation point, presumably due to the diurnal cycling mentioned previously.

Carbon Dioxide Uptake Rates

Carbon dioxide uptake rates (mg $CO_2 \cdot dm^{-2}\ hr^{-1}$) did not stabilize until approximately 48 hours after the leaf was sealed into the chamber

as described in the adaptation section. Similar, though less severe fluctuations were observed when thermoperiods were changed. No data were collected until CO_2 uptake rates had stabilized.

The CO_2 uptake data of Experiments 5, 6, 8 and 9 were obtained while holding night temperature constant and varying the day temperatures. The data of Experiment 7 were obtained at a 35 C day temperature while varying the night temperature. The plant used for the 15 and 20 C constant night temperature studies was grown in nutrient solution. The plant used for the 25, 30 and 35 C constant night temperature studies was grown in sand as was the plant used for the constant 35 C day temperature. Although the data of Table V and VI represent three plants and two methods of culture the results were remarkably consistent.

TABLE V

EFFECT OF THERMOPERIOD ON NIGHT CO_2 UPTAKE BY AN ATTACHED PINEAPPLE 'D' LEAF EXPRESSED AS A PERCENT OF THE TOTAL UPTAKE FOR 24 HOURS

Night Temperature, C	Day Temperatures, C						
	15	20	25	30	35	35[a]	40
15	6	23	30	42	58	77	b
20	b	22	29	33	56	58	b
25	b	0.5	- 0.6	23	56	42	86
30	b	b	b	- 0.4	27	18	73
35	b	b	b	b	- 7.6	3	b

[a]The data were collected by holding day temperature and varying night temperature. All other data were obtained by holding night temperature constant and varying day temperature.

b - No data were collected at these thermoperiods.

TABLE VI

EFFECT OF THERMOPERIOD ON CO_2 UPTAKE (mg dm^{-2}) BY AN ATTACHED PINEAPPLE 'D' LEAF

Temperature, C		CO_2 uptake, mg dm^{-2}		
Night	Day	Night	Day	Whole Cycle
15	15	3.7	49.7	53.2
	20	14.6	49.2	63.8
	25	18.4	42.0	60.3
	30	21.2	26.8	50.3
	35	20.5	15.2	36.0
20	20	16.2	74.6	91.2
	25	23.8	58.2	82.1
	30	20.5	41.6	62.0
	35	27.6	14.6	43.5
25	20	1.1	92.6	93.1
	25	- 0.6	102.6	102.0
	30	16.6	54.2	70.9
	35	21.1	16.4	37.6
	40	24.7	4.0	28.8
30	30	- 0.5	110.2	109.7
	35	13.6	36.6	50.0
	40	19.8	10.8	29.7
35	35	- 4.8	61.2	57.1
15	35	19.9	6.0	25.9
20		19.4	14.0	33.4
25		16.4	22.6	39.0
30		8.2	37.2	45.4
35		1.2	40.0	41.2

The effect of ΔT on the percent uptake in the dark was dominant over the effects of day or night temperature as shown by the results presented in Table V. With the exception of the 20 C day-20 C night thermoperiod, CO_2 uptake in the dark, expressed as a percent of the total uptake over 24 hours, was near zero or negative at ΔT's of 0 or -5 C. As ΔT was increased the percent uptake during the night increased

(Table V). The greatest uptake in the dark, expressed as a percent of the total for the whole cycle, was 86 percent with a 40 C day and a 25 C night thermoperiod (ΔT = 15 C).

Carbon dioxide uptake (mg dm^{-2}) for the night, the day and the whole cycle are shown in Table VI. Dark fixation of CO_2 increased with increasing day temperature at a given night temperature. Maximum dark fixation of CO_2 was measured at 20 C, but as long as ΔT was at least 10 C, approximately 20 mg CO_2 dm^{-2} were fixed at night temperatures from 15 to 30 C. No net dark fixation was measured at the constant temperatures. Uptake of CO_2 in the light increased with increasing day temperature at the constant temperatures. However, with night temperature held constant, CO_2 uptake in the light decreased with increasing day temperature and increasing ΔT. Total CO_2 uptake rates were also affected by ΔT, day, and night temperatures (Table VI). Total uptake was greatest at a constant temperature of 30 C. At a given day or night temperature with the counterpart night or day temperature varying, maximum total uptake corresponded with the constant thermoperiods (Table VI). Total CO_2 uptake was markedly reduced by day temperatures of 35 C and above.

Hourly CO_2 uptake rates are shown in Figures 5 through 10. With the exception of the 35 C thermoperiod, the results of Figure 5 are the constant temperature data also shown in Figures 6 through 9. The data of Figures 6 through 10 are for separate experimental runs on a single leaf.

Hourly CO_2 uptake rates for constant temperatures ranging from 15 to 35 C are shown in Figure 5. A sharp increase in the rate of uptake

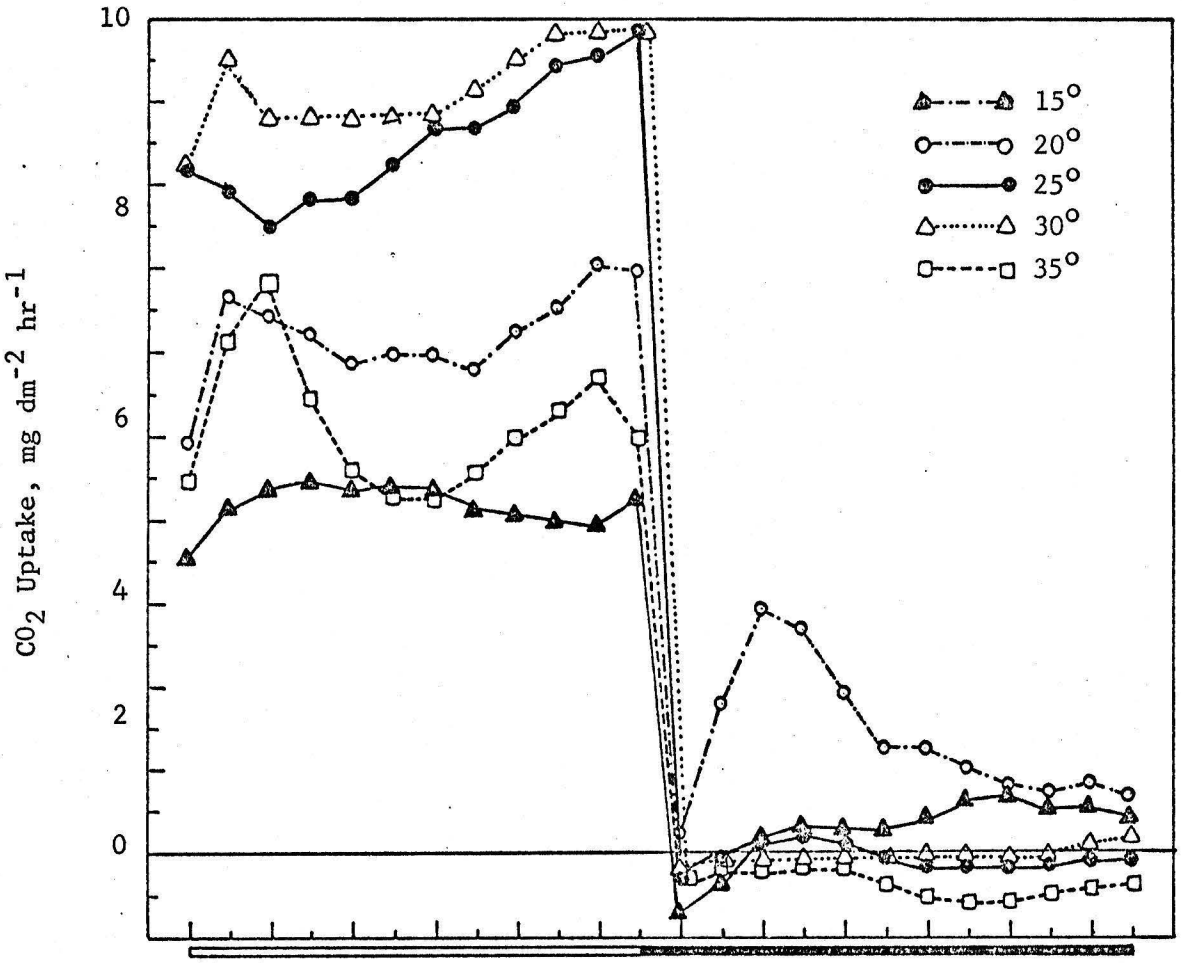


FIGURE 5. HOURLY CO_2 UPTAKE RATES OF AN ATTACHED PINEAPPLE 'D' LEAF AT CONSTANT TEMPERATURES.

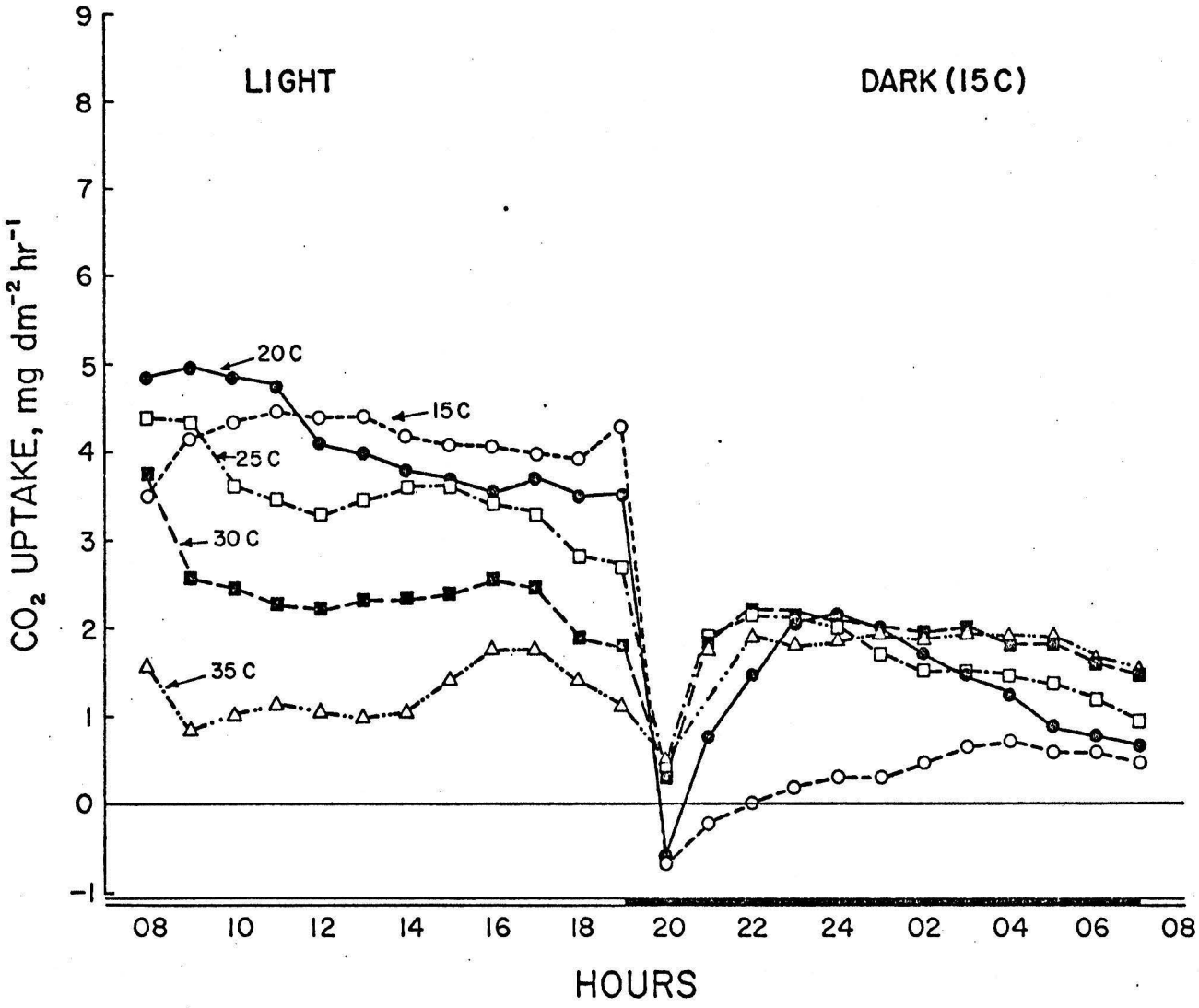


FIGURE 6. HOURLY CO_2 UPTAKE RATES OF AN ATTACHED PINEAPPLE 'D' LEAF AT SEVERAL LIGHT PERIOD TEMPERATURES AND A CONSTANT DARK PERIOD TEMPERATURE OF 15 C.

FIGURE 7. HOURLY CO_2 UPTAKE RATES OF AN ATTACHED PINEAPPLE 'D' LEAF AT SEVERAL LIGHT PERIOD TEMPERATURES AND A CONSTANT DARK PERIOD TEMPERATURE OF 20 C.

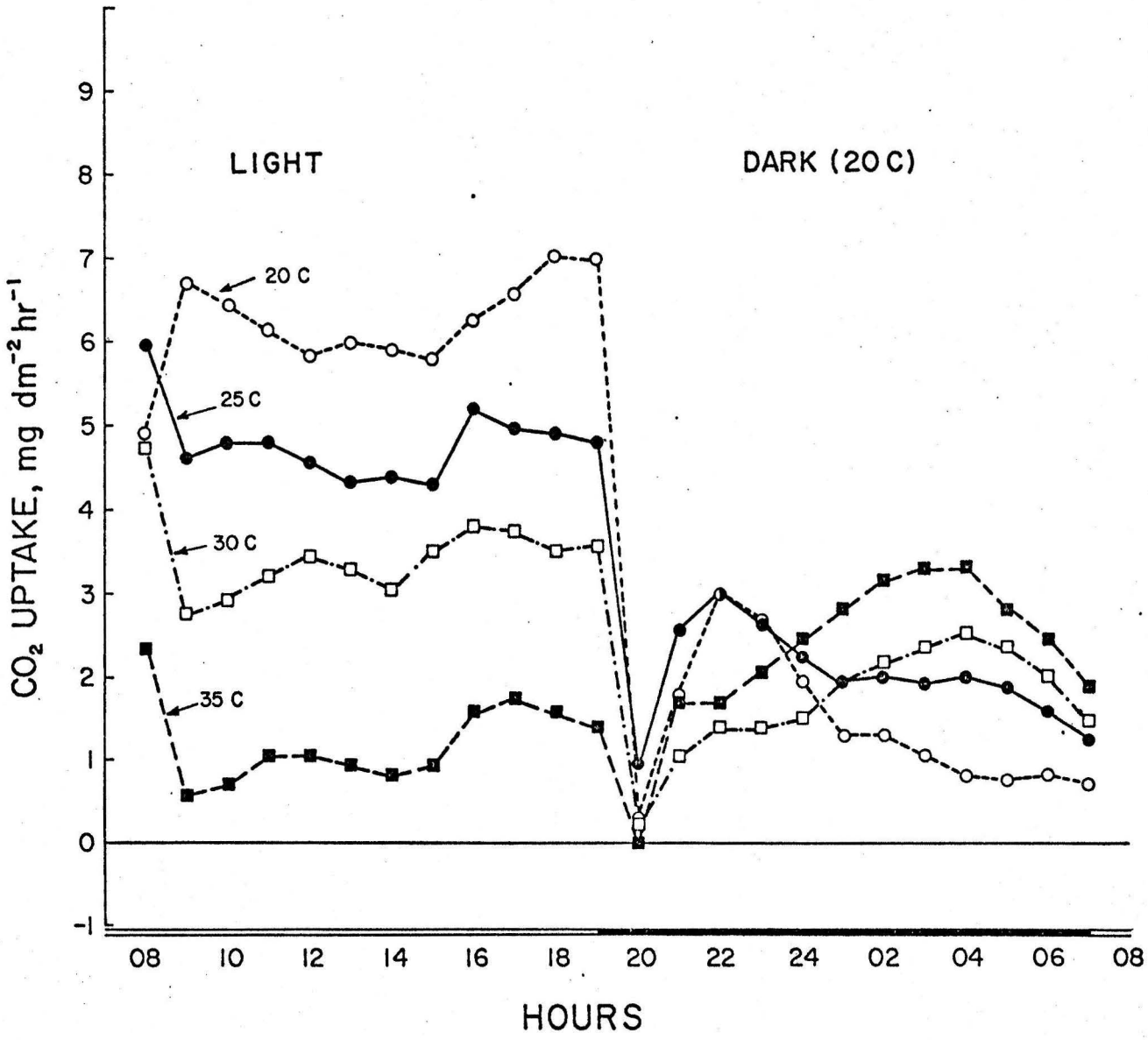

LIGHT
DARK (20 C)
20 C
25 C
30 C
35 C
CO_2 UPTAKE, mg $dm^{-2} hr^{-1}$
9
8
7
6
5
4
3
2
1
0
-1
08
10
12
14
16
18
20
22
24
02
04
06
08
HOURS

FIGURE 8. HOURLY CO_2 UPTAKE RATES OF AN ATTACHED PINEAPPLE 'D' LEAF AT SEVERAL LIGHT PERIOD TEMPERATURES AND A CONSTANT DARK PERIOD TEMPERATURE OF 25 C.

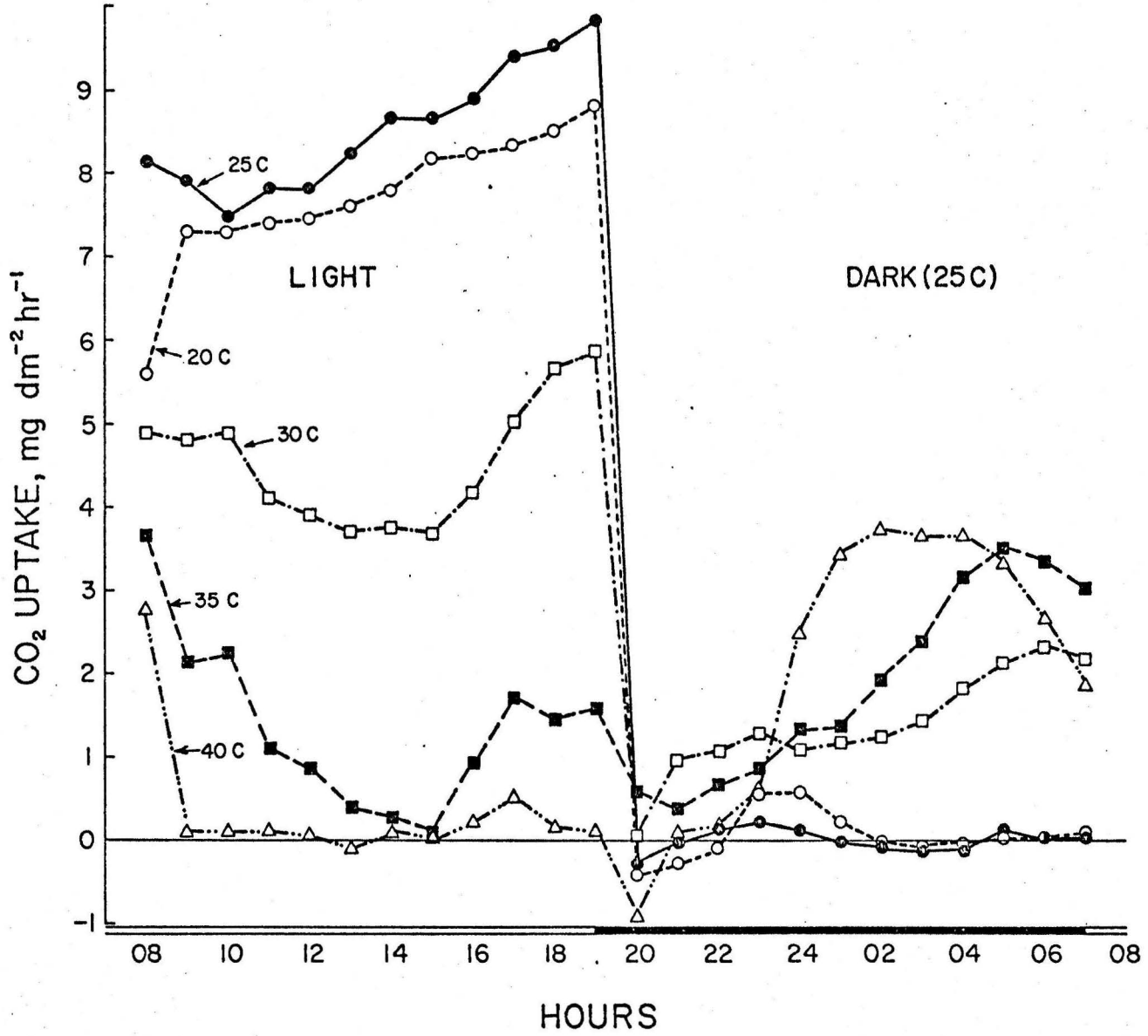

25 C
20 C
30 C
35 C
40 C
LIGHT
DARK (25 C)
CO_2 UPTAKE, mg dm^{-2} hr^{-1}
9
8
7
6
5
4
3
2
1
0
-1
08
10
12
14
16
18
20
22
24
02
04
06
08
HOURS

FIGURE 9. HOURLY CO_2 UPTAKE RATES OF AN ATTACHED PINEAPPLE 'D' LEAF AT SEVERAL LIGHT PERIOD TEMPERATURES AND A CONSTANT DARK PERIOD TEMPERATURE OF 30 C.

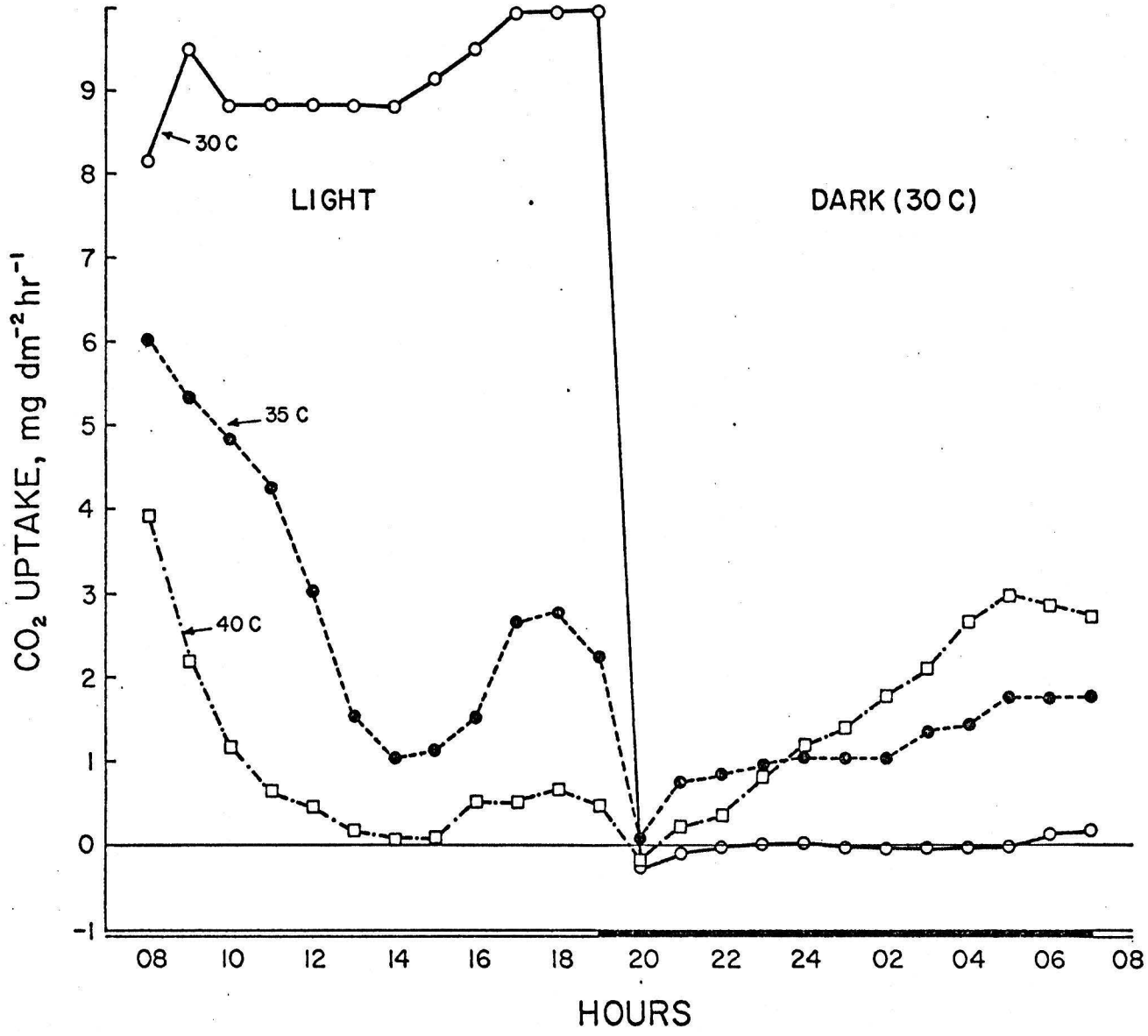
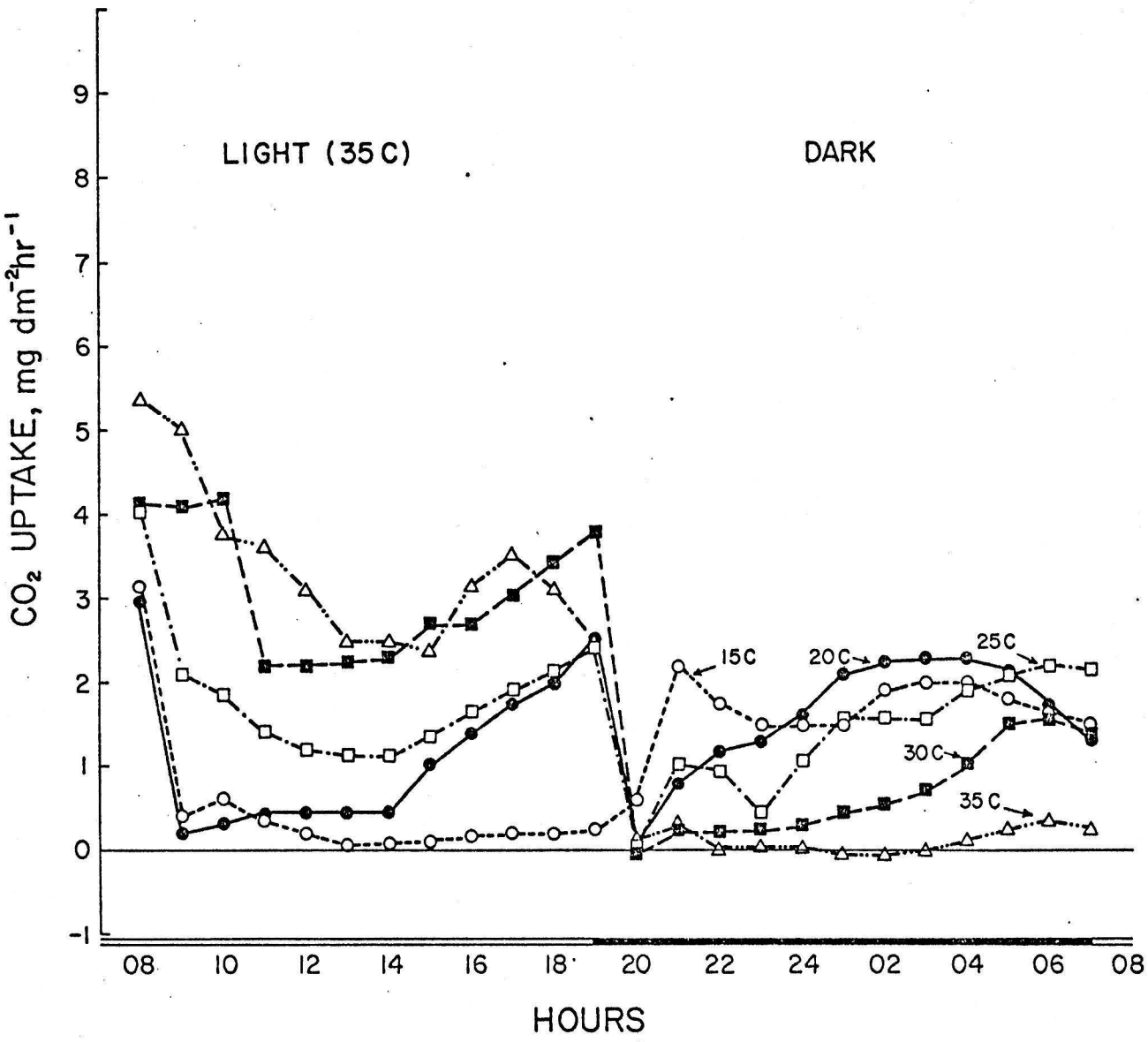

30 C
LIGHT
DARK (30 C)
35 C
40 C
CO_2 UPTAKE, mg $dm^{-2} hr^{-1}$
9
8
7
6
5
4
3
2
1
0
-1
08
10
12
14
16
18
20
22
24
02
04
06
08
HOURS

FIGURE 10. HOURLY CO_2 UPTAKE RATES OF AN ATTACHED PINEAPPLE 'D' LEAF AT A CONSTANT LIGHT PERIOD TEMPERATURE AND SEVERAL DARK PERIOD TEMPERATURES.


LIGHT (35 C)
DARK
CO_2 UPTAKE, mg $dm^{-2}hr^{-1}$
9
8
7
6
5
4
3
2
1
0
-1
15C
20C
25C
30C
35C
08
10
12
14
16
18
20
22
24
02
04
06
08
HOURS

during the first hour of the day (0700-0800 hrs.) was observed at all temperatures except 25 C. As the temperature was increased from 15 to 30 C in 5 C increments there was an increase in the rate of CO_2 uptake during the day. The maximum rate of uptake was measured at 30 C. A sharp decline was observed at 35 C.

The light and dark uptake rates at constant temperatures of 25 and 30 C (Figure 5 and Table VI) were very similar and probably any difference between them was not significant. The 35 C thermoperiod showed a net loss of 4.8 mg CO_2 dm^{-2} for the night (Table VI). The rate of dark fixation was maximal at 20 C, a result that coincides well with the optimum temperature of 20 C reported for dark CO_2 fixation (Bennet-Clark, 1933).

Several general trends are evident in the data shown in Figure 6 through 10. Hourly CO_2 fixation rates during the day were highest where ΔT was zero or negative. At thermoperiods where ΔT was at or near zero, dark fixation was very temperature dependent. Where the dark period temperature was 20 or 15 C, some dark fixation was measured at all thermoperiods. When the dark temperature was increased to 25 C or above, little or no dark fixation occurred and in some cases a net loss was obtained when ΔT was zero. As ΔT was increased, the percent of CO_2 fixed in the dark increased (Table V) and the rate of uptake during the day decreased (Figures 6 through 10). The net result was a decrease in total uptake for the 24 hour period (Table VI). Where ΔT was 5 C or greater, relatively large amounts of CO_2 were fixed in the dark at temperatures ranging from 15 to 30 C. The result is in contrast to

published literature where optimum temperatures for dark fixation have been reported to be at or below 20 C (Brandon, 1967; Bennet-Clark, 1933).

The T also affected the pattern of CO_2 fixation in the dark. When the rate of CO_2 fixation reached a maximum early in the dark period (e.g. Figure 7, 25 C day-20 C night thermoperiod) the rate usually declined throughout the balance of the night. Conversely, when the rate of uptake was low early in the dark period (e.g. Figure 7, 35 C day-20 C night thermoperiod) the rate of uptake continued to increase through the greater part of the night.

In all cases the greatest increase in uptake rates occurred during the first hour of the light period (Figures 5 through 10). No evolution of CO_2 was observed during the first few minutes following the onset of day. However, with the onset of night a burst of CO_2 was observed and is indicated in the sharp drop in uptake rates in the graphs of Figures 5 through 10. The magnitude and the length of time of the burst depended upon the specific thermoperiod. The magnitude and length of time were greatest at a day-night thermoperiod of 40 - 25 C and least at a day-night thermoperiod of 25 - 20 C.

To ascertain if the pattern of CO_2 uptake was the result of treatment (thermoperiod) or the method in which the treatment was applied, in one experiment the day temperature was held constant while the night temperature was varied (Figure 10 and Table V). The results were similar and the same trends were observed as in the constant night-varying day temperature experiments. The day uptake rates were greatest when ΔT was zero or negative and decreased as ΔT decreased.

The effects of continuous light on CO_2 exchange by a pineapple leaf are presented in Table VII. The study was run for 72 hours at a constant temperature of 25 C. The data (continuous light) are for three consecutive days after the plant became adapted to the environment, whereas the data for the 12 hour photoperiod include the period of change over from continuous light to a 12 hour photoperiod. The hourly uptake rate was fairly steady for the continously lighted plant. Although the data did not show the large day-night variations of a 12 hour photoperiod, the total CO_2 uptake was approximately the same for both conditions (Table VII).

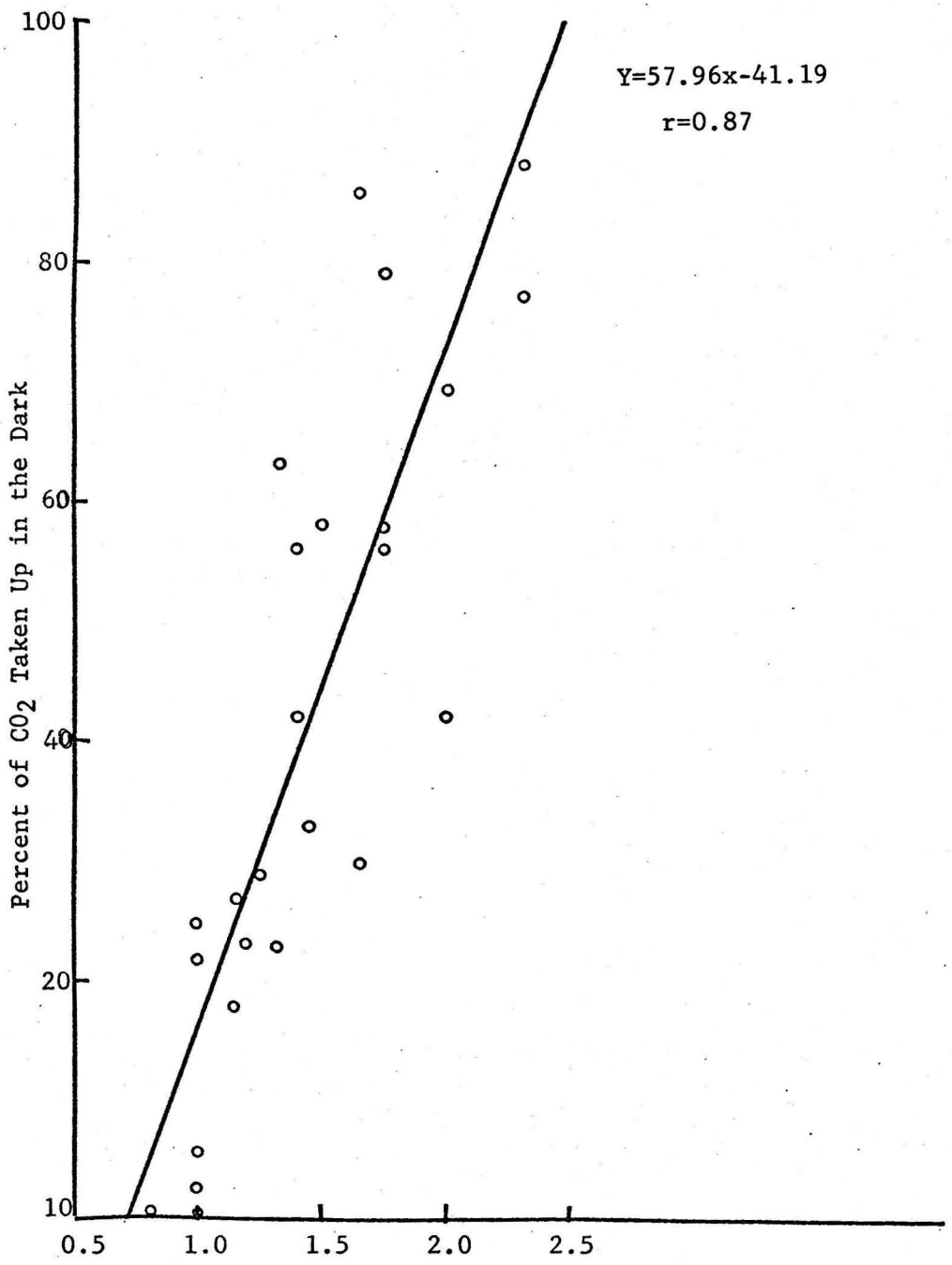
TABLE VII

CO UPTAKE (mg dm^{-2} 24 hr^{-1}) OVER 72 HOURS BY A CONTINUOUSLY LIGHTED PLANT AND THE SAME PLANT WITH A 12 HOUR PHOTOPERIOD. BOTH STUDIES AT 25 C

	CO_2 Uptake, mg dm^{-2}							
	Continuous Light			12 Hour Photoperiod				
Time	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 4	Day 5
0700 to 1800	24.16	27.67	25.38	29.25	37.73	43.40	42.99	42.35
1800 to 0600	26.79	27.43	27.97	- 2.81	2.63	12.64	11.47	14.16
0700 to 0600	50.95	55.10	53.35	26.44	40.36	56.04	54.46	56.51

It is quite obvious from the results shown in Figures 5 through 10 and Tables V and VII that the percent of the total uptake that occurred in the dark was very temperature dependent. Not only is the percent dark uptake dependent on absolute temperature (Figure 5) but also on ΔT. A regression of percent dark uptake on the ratio $\frac{\text{day temperature}}{\text{night temperature}}$ had a highly significant correlation coefficient of 0.87 (Figure 11). The

FIGURE 11. THE REGRESSION OF PERCENT OF CO_2 UPTAKE IN THE DARK ON THE RATIO $\frac{\text{DAY TEMPERATURE}}{\text{NIGHT TEMPERATURE}}$.


Y=57.96x-41.19
r=0.87
100
80
60
40
20
10
0.5
1.0
1.5
2.0
2.5
Percent of CO_2 Taken Up in the Dark
Ratio Day Temperature/Night Temperature

regression included both constant day temperatures with varying night temperatures and constant night temperatures with varying day temperatures. Also included are six points from a plant that had been under water stress of unknown magnitude.

To evaluate the effects of prolonged water stress on CO_2 uptake rates two series of thermoperiods (15 and 20 C night temperatures) were run with a plant that had been subjected to a water stress for sufficient time to collapse the water storage tissue. A comparison of Figures 12 and 13 with 5 and 6 shows that the CO_2 uptake patterns and rates were quite different from the uptake patterns and rates of non-stressed plants. In the stressed plants, only small amounts of CO_2 were taken up in the light even when ΔT was 0 (Figure 13). The hourly uptake rates as well as total uptake of the stressed plants were considerably less than the well watered plants (Figures 5, 6, and 12, 13). The percent of the total CO_2 taken up in the dark was much greater for the water stressed plants (Table VIII) than for non-stressed plants at the same thermoperiods.

TABLE VIII

NIGHT CO_2 UPTAKE EXPRESSED AS A PERCENT OF THE TOTAL UPTAKE BY NORMAL AND WATER STRESSED PLANTS

Night Temp. C	Day Temperature, C 25	30	35
15	30	42	58
	80*	69*	88*
20	29	33	56
	60*	58*	79*

*Water Stressed Plants

FIGURE 12. HOURLY CO_2 UPTAKE RATES AT THREE LIGHT PERIOD TEMPERATURES AND A CONSTANT DARK PERIOD TEMPERATURE OF 15 C FOR A WATER STRESSED ATTACHED PINEAPPLE 'D' LEAF.

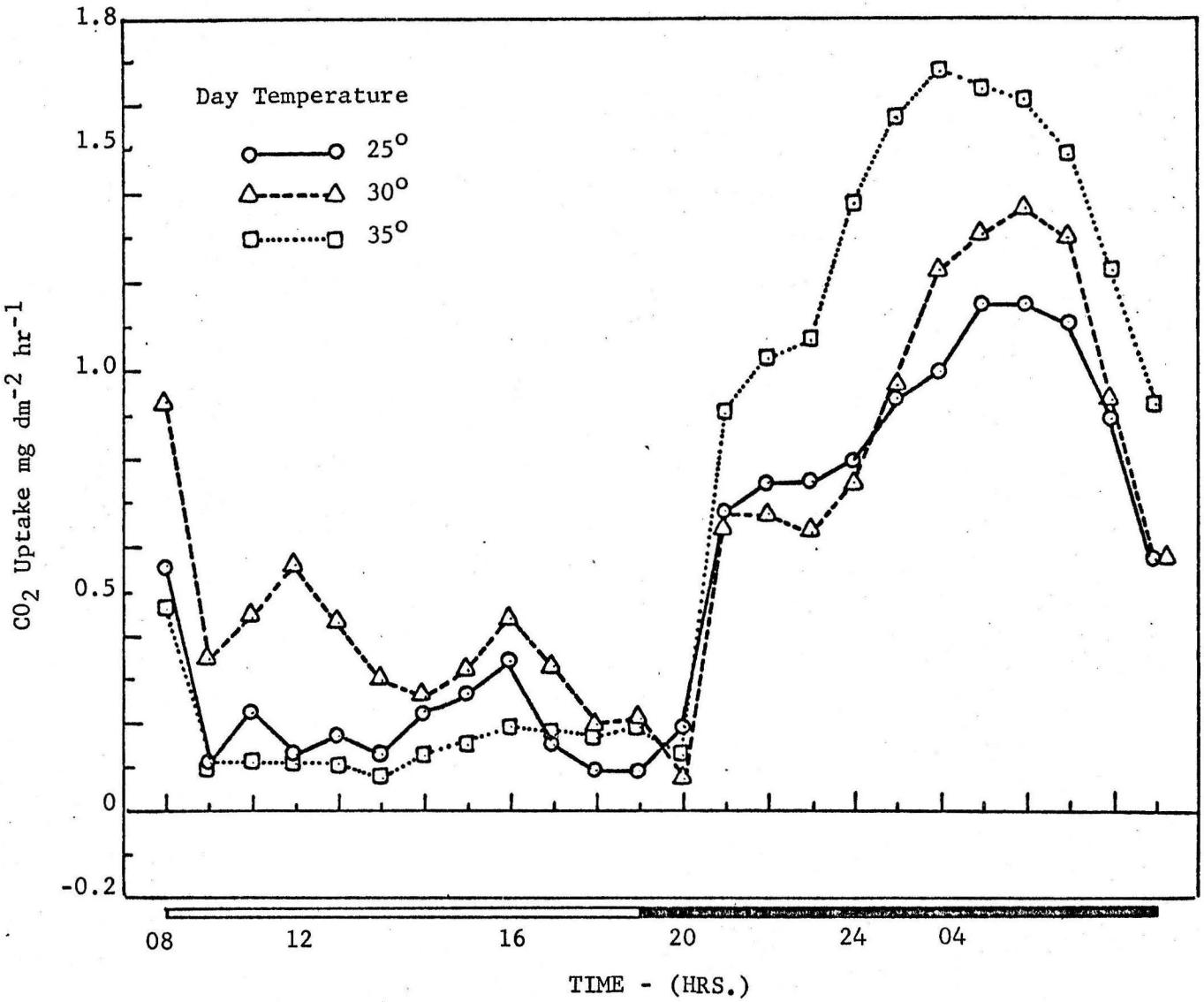
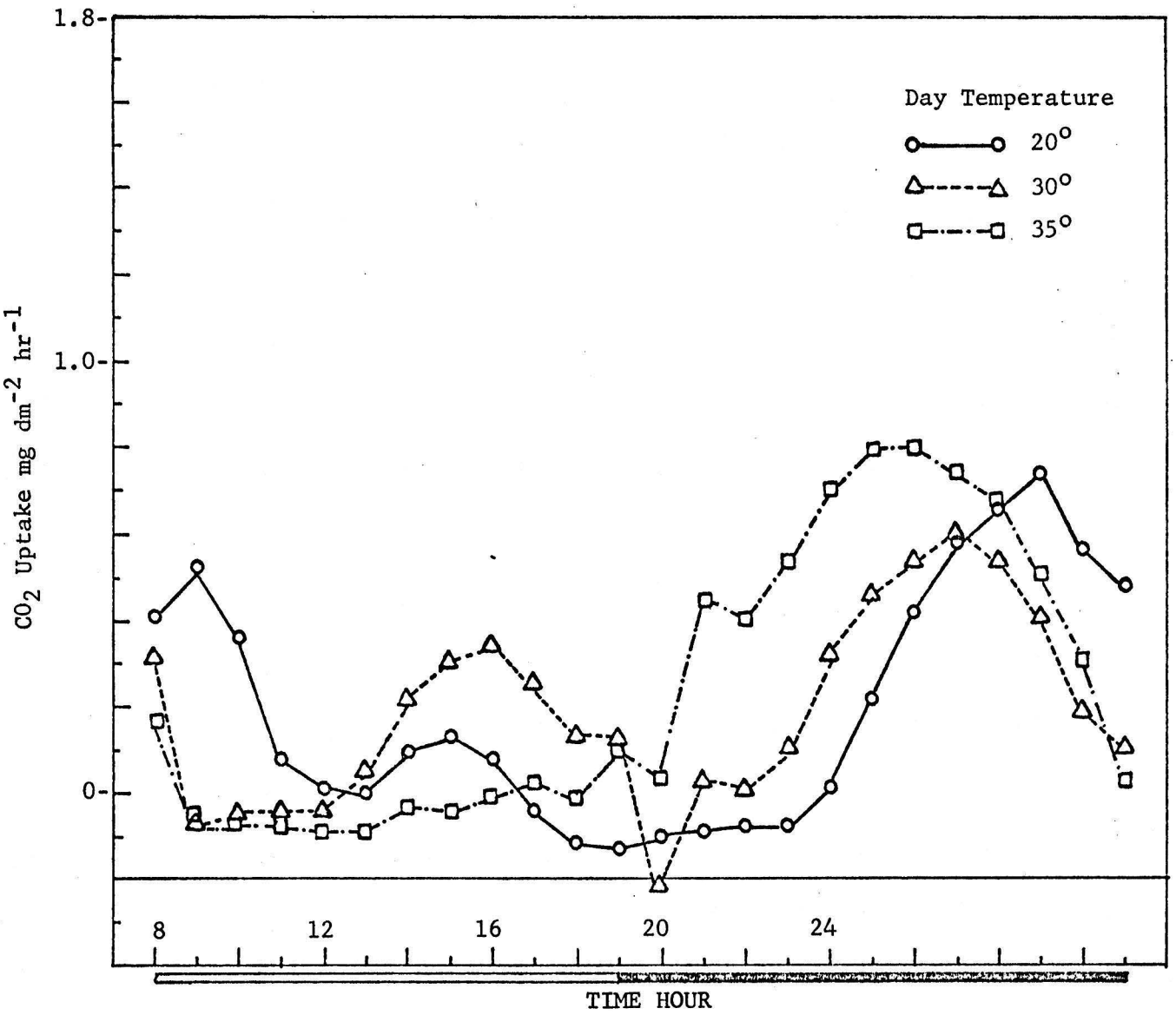

Day Temperature
25°
30°
35°
1.8
1.5
1.0
0.5
0
-0.2
CO_2 Uptake mg dm^{-2} hr^{-1}
08
12
16
20
24
04
TIME - (HRS.)

FIGURE 13. HOURLY CO_2 UPTAKE RATES AT THREE LIGHT PERIOD TEMPERATURES AND A CONSTANT DARK PERIOD TEMPERATURE FOR AN ATTACHED WATER STRESSED 'D' LEAF.


1.8
1.0
0
CO_2 Uptake mg dm^{-2} hr^{-1}
Day Temperature
20°
30°
35°
8
12
16
20
24
TIME HOUR

DISCUSSION AND CONCLUSIONS

Adaptation to the Chamber

Most research workers recognize that when the photosynthetic rate of a plant is to be measured in a controlled environment, a period of adaptation to the environment is required. Researchers measuring photosynthetic rates of mesophytic plants have used adaptation periods of one-half to one hour. Neales (1970) implied that six days were required to establish "-- the characteristic rhythms of CO_2 and water vapor exchange --" for the xerophyte Agave americana. In this study, depending on the pretreatment, from one to nine days were required for the plant to become adapted to a new environment.

For at least the first 24 hours that a plant was in the chamber the CO_2 exchange pattern was not uniform, after which a stable diurnal pattern followed. During the first day the plant responded in a manner similar that described by Joshi, et al. (1965); i.e. evolution of CO_2 in the light and CO_2 uptake during the night. This behavior can be seen from the graph of the continuous equilibrium between CO_2 intake and CO_2 evolution ($E\ CO_{2i}/CO_{2e}$). When the initial CO_2 concentration of the chamber was 300 ppm and CO_2 was neither removed or supplied, the plant evolved CO_2 in excess of 575 ppm in the light and took up CO_2 in the dark (Figure 2A). However, commencing with the second day the CO_2 concentration never exceeded 300 ppm and finally stabilized at a maximum value of 145 ppm CO_2 in the light and 20 ppm CO_2 in the dark (Figure 2B). This nonuniform diurnal pattern of CO_2 uptake and evolution was noted with all plants when they were first transferred to the leaf chamber.

The transpiration data of Yoder (1969) show that stomatal opening responds more-or-less predictably to thermoperiod. Stomatal opening is closely keyed to the internal CO_2 concentration in mesophytes (Meidner and Mansfield, 1968) and apparently also in xerophytes (Neales, 1970). It is likely that the exposure of a xerophyte to a modified environment results in a shift in rates of light and dark CO_2 fixation and malic acid decarboxylation (Brandon, 1967) which are assumed to determine intercellular CO_2 concentrations. The effect of thermoperiod on the CO_2 compensation points (Table II) indicates that the amount and or the activity of the enzymes carboxydismutase and PEP-carboxylase and the malic enzyme are affected by changing thermoperiod. How this occurs is not evident from the results of this study.

Equilibration Studies - No External CO_2 Supplied

The results of the equilibration studies show that the CO_2 compensation point, as normally defined for mesophyte plants (Downton and Tregunna, 1968; Moss, et al., 1969), has little or no meaning where a xerophytic plant such as pineapple having CAM is concerned. As the results of Figures 3 and 4 and Table I show, any compensation point determined for pineapple would have to be specifically defined with respect to thermoperiod and time of day. The significance of this point, once it has been determined, will be discussed in the following section.

The results of Table I and Figure 3 show that night temperature affects the efficiency of the leaf in refixing respired CO_2 in the dark. The result may be due in part to temperature effects on the activity of the malic enzyme and PEP-carboxylase as described by Brandon (1967). The

difference in the length of time required to reach the minimum E CO_{2i}/CO_{2e} in the dark at 35 and 25 C (Figure 3 B and C) appears to be mainly an effect of day temperature on the maximum value of E CO_{2i}/CO_{2e} measured during the day. The rate of decline in CO_2 concentration in the dark is similar in both sets of data.

The maximum E CO_{2i}/CO_{2e} values obtained during the day (Table I) were also affected by temperature. The results do not seem to be due entirely to temperature effects on the respiration rate. The 50 percent increase in the equilibrium value from 20 to 25 C could be accounted for by respiration. The large increases in E CO_{2i}/CO_{2e} at 30 and 35 C are too great to be accounted for by the expected increases in the respiration rate. The results are not consistent with the compensation point data obtained for pineapple plants which were in normal air the majority of the time. The data contribute little to the understanding of CO_2 metabolism in pineapple but definitely show the lack of a steady state CO_2 compensation point (Downton and Tregunna, 1968) for pineapple.

CO_2 Compensation Points

Carbon dioxide compensation points have been determined on plants for some time but the significance of this measurement is still somewhat open to question. However, it is generally believed that the compensation point gives an indication of:

a. the major pathway by which CO_2 is fixed i.e., the C_4 dicarboxylic acid or C_3 (Calvin) pathway
b. the rate of CO_2 fixation
c. the photosynthetic efficiency
d. the presence or absence of photorespiration.

The only data on the CO_2 compensation point of a xerophyte were reported by Jones and Mansfield (1970) for Bryophyllum.

Carbon dioxide compensation points of pineapple were measured as an aid to understanding how environment affected CO_2 fixation processes in this species. Compensation points approaching zero could indicate that CO_2 was fixed by the C_4 pathway while compensation points above 40 ppm could indicate that CO_2 was being assimilated primarily via the C_3 pathway.

The CO_2 compensation point for each thermoperiod was determined on an adapted plant after the lights were turned on (Table II). All data were collected at the end of an experiment set up to study the effects of a specific thermoperiod on the hourly rate of CO_2 uptake over a 24 hour period.

The results of the compensation point determinations are difficult to interpret. It is not known to what extent the compensation point reflects metabolic processes and to what extent it reflects stomatal closure since measurements of leaf resistance to water vapor diffusion were not made. However, in some cases the compensation point was approached from 0 ppm CO_2 as well as from 300 ppm CO_2 with essentially identical results. This would indicate that the compensation point was unaffected by stomatal closure and that it truly reflected the intercellular space CO_2 concentration.

A generalization that can be made about the results is that the lower the compensation point, the lower the average rate of CO_2 depletion and the longer the time required to reach the compensation point (Tables III and IV). It might be expected that the rate of CO_2 depletion would remain

constant over the entire range of CO_2 concentrations so long as the stomata remain open. However, the closer the compensation point was to zero, the greater would be the CO_2 gradient between the closed circuit system and the atmosphere and the more the effect of very small leaks in the system would be magnified. Also, the average depletion rate (Table IV) does not reflect the observed response at thermoperiods where the CO_2 compensation point approached zero. For these determinations, it was observed that the CO_2 concentration declined very rapidly at first but the rate of decline decreased concomitantly with the CO_2 concentration. This greatly lowered rate of CO_2 depletion at low CO_2 concentrations no doubt was due to the decrease in the number of extractable molecules per unit volume and the magnified effect any minute leaks in the system may have had.

Based on the above considerations, it appears that the CO_2 compensation point data do show a decrease in the activity of enzymes involved in CO_2 fixation or the inhibition of one of the major carboxylation enzymes (probably PEP-carboxylase) as the CO_2 compensation point decreased with increasing ΔT. A specific temperature effect was also evident from the data and similar effects have been reported for other plants (Heath and Orchard, 1957).

Presumably the CO_2 compensation points below 10 ppm obtained at the 20 C day-25 C night and the 25 C day-25 C night thermoperiods indicate CO_2 fixation via the C_4 dicarboxylic acid pathway. Compensation points above 40 ppm would indicate that CO_2 was being fixed primarily via the C_3 or Calvin pathway. However, this leaves the compensation points between 10 and 40 ppm unexplained and no data have been found which show

compensation points in the range of CO_2 concentrations from 95 to 170 ppm. Thus, there seems to be little justification for drawing conclusions about CO_2 fixation pathways for pineapple based on compensation point and metabolic pathway data obtained for mesophytic plants. The only valid conclusion that can be drawn with respect to the data of Table II is that the CO_2 compensation point, as determined in this study, was extremely variable and that variability was due to such factors as the environment temperature and the ΔT and their subsequent effects on dark and light CO_2 fixation.

CO_2 Uptake Rates

It has been reported that CO_2 is liberated from CAM plants when the plants were first transferred from dark to light (Ranson and Thomas, 1960). In the present study with pineapple, this was not observed. As soon as the lights were turned on an immediate increase in the CO_2 uptake rate occurred, reaching a plateau that varied with the specific thermoperiod. After the initial increase the CO_2 uptake rate was fairly steady throughout the day when the plant was under constant temperature regimes (Figure 5). However, when the differential between day and night temperature (ΔT) was not equal to zero a decrease in CO_2 uptake occurred by midmorning (Figures 6 through 10).

An increase in the rate of CO_2 evolution when the lights are turned off has also been reported (Gregory, et al., 1954). The result was interpreted by Ranson and Thomas (1960) as being due to the 'respiratory power' of the leaves. At the onset of night, evolution of CO_2 was detected from pineapple leaves (Figures 5 through 10). The magnitude of the evolution varied with the specific thermoperiod. After the initial

burst of CO_2, the plant either fixed CO_2 or continued to respire CO_2 at lower rates throughout the remainder of the night (Figures 5 through 10).

The evolution of CO_2 associated with the onset of night may have been due to the 'respiratory power' of the leaf but this was probably accentuated by the square wave light pattern and sine wave temperature pattern prevailing in this study. The light intensity in the chamber went from full intensity to complete darkness instantaneously, whereas the temperature decreased slowly to the specific night setting. As soon as the lights were turned off photosynthesis ended, but respiration would continue at rates reflecting the temperature of the leaf in the light. As the internal leaf CO_2 concentration increased a diffusion gradient would be established towards the atmosphere. The higher the temperature the faster the rate of respiration and thus the greater the rate of CO_2 evolution. At thermoperiods where the temperature decreased to a lower night setting the rate of respiration decreased and dark CO_2 fixation increased reversing the diffusion gradient towards the leaf.

CAM type plants are characterized as having a capacity for massive dark fixation of CO_2. The data from this study showed that the night uptake of CO_2 by pineapple depends not only upon absolute temperature, but also upon ΔT. According to the data, dark fixation becomes significant when the plant is subjected to environment which have wide temperature variations between night and day. As the diurnal variation in temperature decreased, the percentage of dark fixation decreased. With pineapple, it was found that in order for dark uptake to exceed uptake in the light the day temperature had to be greater than 30 C and ΔT had to be 15 C. At day temperatures of 40, 35 and 30 C, dark fixation

of CO_2 increased as night temperatures were decreased to 20 C. Dark fixation at 20 C and 15 C was appreciable at all day temperatures. It was presumed that the cooler night temperatures enhanced malate production and storage. Bennet-Clark (1933) reported night temperatures of 20 C or less favored CAM whereas above 30 C, deacidification occurred at night.

The effects of night temperature on hourly rates of CO_2 uptake are shown quite clearly in Figure 10. At a constant day temperature of 35 C, dark fixation and presumably malate synthesis increased as night temperatures decreased from 35 C to 20 C. The uptake rate reached a plateau and then declined before the onset of day. The rates associated with the warmer night temperatures increase more slowly and do not start to decline as soon as the rates at the cooler night temperature (Figure 10). There must be some maximum capacity to fix CO_2 in the dark which is dependent upon photoperiod and light intensity (Seshagiri and Suryanarayanamurthy, 1957), temperature (Bennet-Clark, 1933; Brandon, 1967) and Kent[1] demonstrated an effect of CO_2 concentration. As this maximum level is approached, the dark fixation rate would be expected to decline due to end-product inhibition. A low but steady rate of uptake throughout the night could indicate failure to reach a level where end-product inhibition occurred. The decline in dark CO_2 uptake rates observed at many of the thermoperiods quite possibly was associated with end-product inhibition of malate synthesis.

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[1]Kent, M. J. In the files of the Pineapple Research Institute of Hawaii.

Only two reported dealing with the CO_2 exchange of pineapple leaves as measured by infrared gas analysis are in the literature. Neales, et al. (1968) reported that pineapple assimilated 37 percent of the total CO during the dark period in a 31 C day-16 C night. This agrees well with the 41 percent night uptake found in this study for the comparable 30 C day-15 C night thermoperiod (Figure 6). However, it should be noted that Neales, et al. (1968) used a 16 hour photoperiod, whereas the data presented in Table V and VI represent a 12 hour photoperiod. If the average fixation rates of Neales, et al. are recalculated on the basis of a 12 hour photoperiod, dark fixation accounts for 42 percent of the total uptake. Although the percent of dark uptake in these two studies was approximately equal there was a major difference in the hourly uptake rates. The rates presented by Neales, et al. (1968) increased continuously throughout the night, whereas the data presented in Figure 6 generally showed a progressive increase in the CO_2 fixation rate followed by a decline before morning. The difference may be due in part to the difference in the length of the photoperiod in the two studies. The quantity of malic acid synthesized during the night has been reported to depend upon the amount of photosynthate produced during the day preceeding the dark period (Ranson and Thomas, 1960), the light intensity, and the temperature. One can speculate that the 16 hour photoperiod would produce carbohydrate reserves sufficient to sustain high rates of dark fixation or that the eight hour night was of insufficient length to allow accumulation of malate to its maximum level.

Under constant or near constant thermoperiods, day CO_2 uptake greatly exceeded uptake during the night (Table VI). Even though CAM plants are capable of massive dark CO_2 uptake, the highest night uptake rates never exceeded 3.7 mg CO_2 dm^{-2} hr^{-1}, whereas the maximum day uptake rates exceeded 9 mg CO_2 dm^{-2} hr^{-1}.

Neales, et al. (1968) using a single attached pineapple leaf (leaf age or position not specified) found the highest rates of uptake during the day. The maximum rates reported were approximately 2 mg CO_2 dm^{-2} hr^{-1} with a 30 C day-26 C night thermoperiod and 0.94 mg for a 31 C day-16 C night. In the present study day uptake rates for similar thermoperiods (30-25 and 30-15) were 4.5 and 2.4 mg CO_2 dm^{-2} hr^{-1}. The The rates obtained for this study were considerably higher than those of Neales, et al.(1968). However, since leaf age was not specified in their experiment it is inappropriate to draw any conclusions from comparisons of the two sets of data.

When a pineapple plant was exposed to a constant temperature of 24 C, CO_2 evolution was measured during the day with a net loss of 2.64 mg CO_2 dm^{-2} (Joshi, et al., 1965). The average CO_2 exchange rate they reported was approximately 0.6 mg CO_2 dm^{-2} hr^{-1} for an 11 hour photoperiod. If the data are recalculated for the 24 hour period, the average rate would be 0.3 mg dm^{-2} hr^{-1}. In this study at a constant temperature of 25 C the average uptake rate for an adapted plant in the light was 8.5 mg CO_2 dm^{-2} hr^{-1} and 4.2 mg for the whole cycle. When compared to the uptake rates and patterns of uptake measured in the present study, the rates measured by Joshi et al. were extremely low and the uptake pattern was the inverse of that presented in Figure 8. Joshi,

et al. (1965) did not precondition their plants, but rather collected data immediately after the plant was moved from the greenhouse to the chamber. The results obtained for an unadapted plant (Figure 2) show that a plant evolved CO_2 (above 300 ppm) during the first day in the chamber, with CO_2 uptake beginning early in the night, a result similar to the data presented by Joshi, et al. (1965).

Total uptake, mg dm^{-2} day^{-1}, increased as temperature increased from 15 C to 30 C. Above 30 C there was a sharp reduction in uptake. The total uptake for the 35 C thermoperiod varied from 29 to 55 percent less than for the 30 C thermoperiod, depending upon the night temperature. The reduction was even greater when the temperature was raised to 40 C (Figure 9 and Table VI). The reduction in CO_2 uptake was probably due to temperatures above optimum for the enzyme systems involved in photosynthesis. A factor not investigated was stomatal closure. It can be assumed on the basis of Yoder's (1969) data that under constant temperature regimes, stomata are fully open during the day at constant temperatures as high as 30 C. However, reductions in the transpiration rate at 35 C indicate that as the temperature was increased to 35 or 40 C the stomata may not have been fully open.

The question arises as to what effect day or night uptake has on the other. Bruinsma (1958) pointed out that the brighter the day the greater the maximum acid concentration. Sideris, et al. (1948) and Seshagiri and Suryanarayanamurthy (1957) also noted the same phenomenon. Although not conclusive, some speculation about CO_2 regulation can be made from the data gathered in this study. The data would seem to indicate that CO_2 uptake during the night controls day CO_2 uptake. When

changing thermoperiods to one that enhanced day uptake, a greater change was observed in the day uptake than in the dark uptake while relatively small changes in dark fixation were reflected in large differences in day CO_2 uptake (Table VI).

Arid and semiarid climates are generally characterized by wide diurnal temperature variations and an inadequate water supply. It has been suggested that CAM is an adaptation to a xerophytic environment (Neales, et al., 1968). Under arid or semiarid environments water losses during the day would be reduced as the stomata were closed. Dark fixation of CO_2 as malate could provide a source of CO_2 for photosynthesis.

When CO_2 uptake by a water stressed plant was measured (Figures 12 and 13), CO_2 uptake was maximum during the night and minimum during the day. Under these same conditions well-watered plants sustained considerably higher rates of CO_2 uptake in both the light and dark. The results for the water stressed plants could well represent CO_2 exchange patterns and rates for pineapple subjected to a drouthy environment. If the temperature and moisture regimes are favorable, the pineapple plant will function similarly to mesophytic plants with respect to CO_2 uptake. The one major difference noted between pineapple and mesophytic plants was the almost complete lack of CO_2 evolution during the night for pineapple.

However important the role of dark fixation of CO_2 and the synthesis of malate, it may actually decrease the total CO_2 uptake for the 24 hour period. Compensation point data (Table II) indicate that during the day when malate was transported from the vacuole and decarboxylated it caused an increase in the internal leaf CO_2 concentration which may in turn have

brought about stomatal closure. Kent[2] reported stomatal closure by pineapple with increasing CO_2 concentration. Stomatal closure was also indicated by reduced transpiration rates of Agave americana at CO_2 concentrations of 400 ppm or greater (Neales, 1970).

Although decarboxylation of malate may indirectly reduce photosynthesis through stomatal closure, it may also reduce CO_2 fixation by enzymatic inhibition. A study of the interaction between malate synthesis and dark and light CO_2 fixation in the succulent Bryophyllum tubiflorum (Kluge, 1969; Kluge, 1971) showed that malate inhibited the incorporation of $^{14}CO_2$ into malate and promoted the synthesis of sucrose in the light. When no or low levels of malate were present the $^{14}CO_2$ was incorporated into malate in the light. Kluge (1969, 1971) and Ting (1968) concluded that malate regulated CO_2 fixation by either feed-back or end-product inhibition of PEP-carboxylase. In pineapple, at dark temperatures of 15 and 20 C and at thermoperiods where ΔT was greater than 0, approximately 20 mg CO_2 dm^{-2} were fixed in the dark. Where the day temperatures were greater than 30 C, and dark fixation was appreciable, CO_2 uptake increased rapidly and then declined when the lights were turned on (especially see Figures 7 and 10, 35 C day-20 C night thermoperiod). If malate inhibition of PEP-carboxylase occurred in pineapple where dark CO_2 fixation was appreciable, the inhibition was progressive with time after the lights were turned on. Thus the transport of malate from the storage site to the site where inhibition of PEP-carboxylase would occur is apparently light mediated. This is evident from the

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[2]Kent, M. J. In the files of the Pineapple Research Institute of Hawaii.

results shown in several of the graphs (see especially Figure 9, 35 C day-30 C night thermoperiod) where the rate of dark fixation was high or even increasing when the lights were turned on. When the lights came on the CO_2 uptake rate increased briefly and then declined.

A serious contradiction to a malate-inhibition hypthesis is, however, evident from the results shown in Figures 6 and 7. At almost all thermoperiods with dark temperatures of 15 and 20 C, dark fixation was very high but had no apparent effect on light uptake at the constant or near constant thermoperiods. The linear relationship between the ratio

$$\frac{\text{day temperature}}{\text{night temperature}}$$

and the percent of CO_2 fixed in the dark (Figure 11) indicates that in pineapple at least, the thermoperiod effect overrides the effect of temperature on the balance between PEP-carboxylase and the malic enzyme (Brandon, 1967) on the one hand and PEP-carboxylase inhibition by malate (Kluge, 1969; Kluge, 1971) on the other. Since all of the metabolic events mentioned above are temperature dependent, if they occur in pineapple it is probable that the array of temperatures examined interact with the metabolic events to the extent that a unifying hypothesis is not possible without further research.

On the assumption that the thermoperiod effects on CO_2 uptake can be extrapolated to the field environment, the different uptake patterns observed at the different temperature regimes could very well explain the difference in time that has been reported for fruiting to occur in various environments (See Introduction). The average diurnal fluctuation in temperature for Ivory Coast is 2 to 3 C and the annual temperature variation is about the same. Hawaii (Oahu) has diurnal variations in

temperature of about 10 C, while Swaziland has even greater temperature variation. In the Introduction it was pointed out that fruiting varies from 15 months in Ivory Coast to as much as three years in Swaziland. No ratoon crop is obtained in Ivory Coast while ratooning is possible in both Hawaii and Swaziland. However, ratooning requires approximately one year more in Swaziland than in Hawaii. Available light and average temperature may account for some of the above mentioned variation, but the CO_2 uptake studies indicate that diurnal temperature variation could be the dominant factor in the growth rate of pineapple in these different environments.

Transpiration Ratios

The low transpiration ratio or high water use efficiency of pineapple (Ekern, 1965; Joshi, et al., 1965; Neales, et al., 1968) has previously been attributed to stomatal closure when evaporative demands were greatest. Walker (1962) in his review of CAM speculated on the advantages of such an environmental adaptive mechanism. The fixation of CO_2 during the night when stomata are open and the utilization of this CO_2 for photosynthesis during the day when stomata are closed would serve as an invaluable aid to survival in a xerophytic environment (Neales, et al., 1968).

Joshi, et al. (1965) calculated a transpiration ratio of 50.3 for pineapple in their experiment and showed that the data of Sideris and Krauss (1928) gave a transpiration ratio of 55. Neales, et al. (1968) reported transpiration ratios of 53 for an attached pineapple leaf.

No transpiration data were collected in the present experiment. However, a study on the effects of thermoperiod on transpiration of

pineapple was conducted by Yoder (1969). The plants were grown with the same nutrient solution as used in this study and many of the thermoperiods examined were identical. The data of Yoder (1969) were obtained from small pineapple plants (11 to 18 dm^2 of leaf area) whereas the data from the present study were obtained from single 'D' leaves of approximately 2 dm^2 area. Transpiration ratios were calculated using both sets of transpiration data where more than one result was obtained for a specific thermoperiod.

The effects of thermoperiod on CO_2 uptake rates (Table VI) showed striking similarity to the effects of thermoperiod on transpiration rates (Table IX). The maximum transpiration rates (Yoder, 1969) and maximum CO_2 fixation rates (Table VI) were measured at a constant temperature of 30 C. Transpiration and CO_2 fixation rates were greatest in the light when $\Delta T=0$ and greatest in the dark when ΔT was large. The transpiration ratios (Table IX), which were little affected by thermoperiod, are similar to the results of Joshi, et al. (1965) and Neales, et al. (1968). Assuming the comparisons are representative, the water use efficiency of pineapple remains more-or-less constant over a very wide range of environments. Thus in environments with little diurnal variation such as Ivory Coast, the data of Yoder (1969) show that higher water use rates could be expected but the results of the two studies would indicate that water use efficiency would remain very high.

TABLE IX

EFFECTS OF THERMOPERIOD ON THE TRANSPIRATION RATES AND THE TRANSPIRATION RATIOS OF PINEAPPLE

Temperature		Transpiration Rate[a] mg dm^{-2} hr^{-1}			Transpiration Ratio[b]		
Light	Dark	Light	Dark	24 Hour	Light	Dark	24 Hour
35	35[c]	87	52	70	25	--	43
	35[c]	145	62	103	42	--	63
	30	124	52	88	60	67	61
	25	64	70	67	68	58	61
	20	75	95	85	90	60	71
	15	61	42	52	70	36	51
30	30	209	75	142	33	--	45
25	25[c]	128	51	89	22	--	31
	25[c]	169	67	118	29	--	41
	20	120	80	100	36	59	42
	15	97	78	88	41	75	52

[a]Transpiration rates are those measured by Yoder, 1969.

[b]Transpiration ratios were calculated by dividing the results shown in Table VI by the ratio $\frac{\text{molecular wt. } CO_2}{\text{molecular wt. } CH_2O}$ and dividing the result into the transpiration rate.

[c]Duplicate sets of transpiration rates were obtained at the specified thermoperiod.

SUMMARY

An automated system was built for the measurement of CO_2 uptake of a single leaf over periods of several days. CO_2 was maintained at a preset concentration by automatically injecting or removing the gas from the system. Utilizing the system, the effects of several thermoperiods on the CO_2 compensation points and CO_2 uptake rates of the youngest fully expanded pineapple leaf were measured. The photoperiod was maintained at 12 hours. The plants used in the study were grown in both sand and solution culture. Three to nine days of adaptation at a specific thermoperiod were required to obtain uniform uptake rates.

CO_2 compensation points, determined just after the lights were turned on, varied from a high of 170 ppm at a 35 C light-15 C dark thermoperiod to 0 ppm at a constant temperature of 25 C. Values for the other thermoperiods generally decreased as the difference between the light and dark temperature was decreased. The results indicate involvement of different enzyme systems in CO_2 fixation at the different thermoperiods. The CO_2 equilibrium, with no CO_2 supplied, was monitored continuously for periods up to 4 days. At 20 C in the dark essentially all the CO_2 was extracted from the sealed chamber. Extraction efficiency of the leaf in the dark decreased with increasing temperature. In the light at a 35 C light-30 C dark thermoperiod, the CO_2 concentration ranged from 69 to 200 ppm. Lower maxima were measured at a dark temperature of 20 C and at a 25 C light-20 C dark thermoperiod. No diurnal cycling in the CO_2 concentration was observed in continuous light or dark at constant temperatures of 20 or 25 C.

When the CO_2 concentration was maintained at 300 ppm, CO_2 uptake was maximal at the constant thermoperiods and ranged from 53 to 109 mg dm^{-2} for 24 hours as temperature was increased from 15 to 30 C. The total amount of CO_2 fixed per 24 hours decreased to 50 percent of the maximum at a constant temperature of 35 C. At a constant dark temperature, the mg of CO_2 fixed in the light and total mg for 24 hours decreased as the temperature of the light period was increased from 15 to 40 C. When the difference between the light and dark temperatures was 5 C or greater, 15 to 20 mg CO_2 dm^{-2} were fixed in the dark even at temperatures of 30 C. The percent of CO_2 fixed in the dark ranged from 0 at a constant temperature of 25 C to 86 at a 40 C light-25 C dark thermoperiod. Total CO_2 fixation decreased as the percent of CO_2 fixed in the dark increased. The dominant factor determining CO_2 fixation rates of pineapple under these conditions appeared to be the amount of CO_2 fixed in the dark. Dark fixation was determined primarily by the difference between the light and dark temperatures and, to a lesser degree, by the actual temperature.

APPENDICES

APPENDIX A

TABLE I. COMPOSITION OF NUTRIENT SOLUTION

NITROGEN SOURCE	g/l NITROGEN STOCK SOLN	ml NITROGEN STOCK SOLN/ 1 APPLIED SOLN
NH_4NO_3	39.98	10
MACRO-NUTRIENTS	**g/l MACRO STOCK SOLN**	**ml MACRO-NUTRIENT STOCK/1 APPLIED SOLN**
KH_2PO_4	65.85	1
K_2SO_4	44.60	2
$MgSO_4.7H_2O$	202.80	1
$CaCl_2.2H_2O$	55.40	1
MICRO-NUTRIENTS	**g/l MICRO STOCK SOLN**	
H_3BO_4	2.86	
$MnCl_2.4H_2O$	1.81	
$CuSO_4.5H_2O$	0.08	
$ZnSO_4.7H_2O$	0.22	
$NaMoO_4$	0.50	
Chelate Fe 138	25.00	

ALL PLANTS RECEIVE 0.25 ml MICRO-NUTRIENT SOLUTION PER LITER OF APPLIED SOLUTION

APPENDIX B

FIGURE 1. DIAGRAM OF THE CONTROLLED ENVIRONMENT CHAMBER AND REFRIGERATED WATER BATH.

a. 500 watt incandescent flood lamps

b. Lamp cooling bath and infrared filter

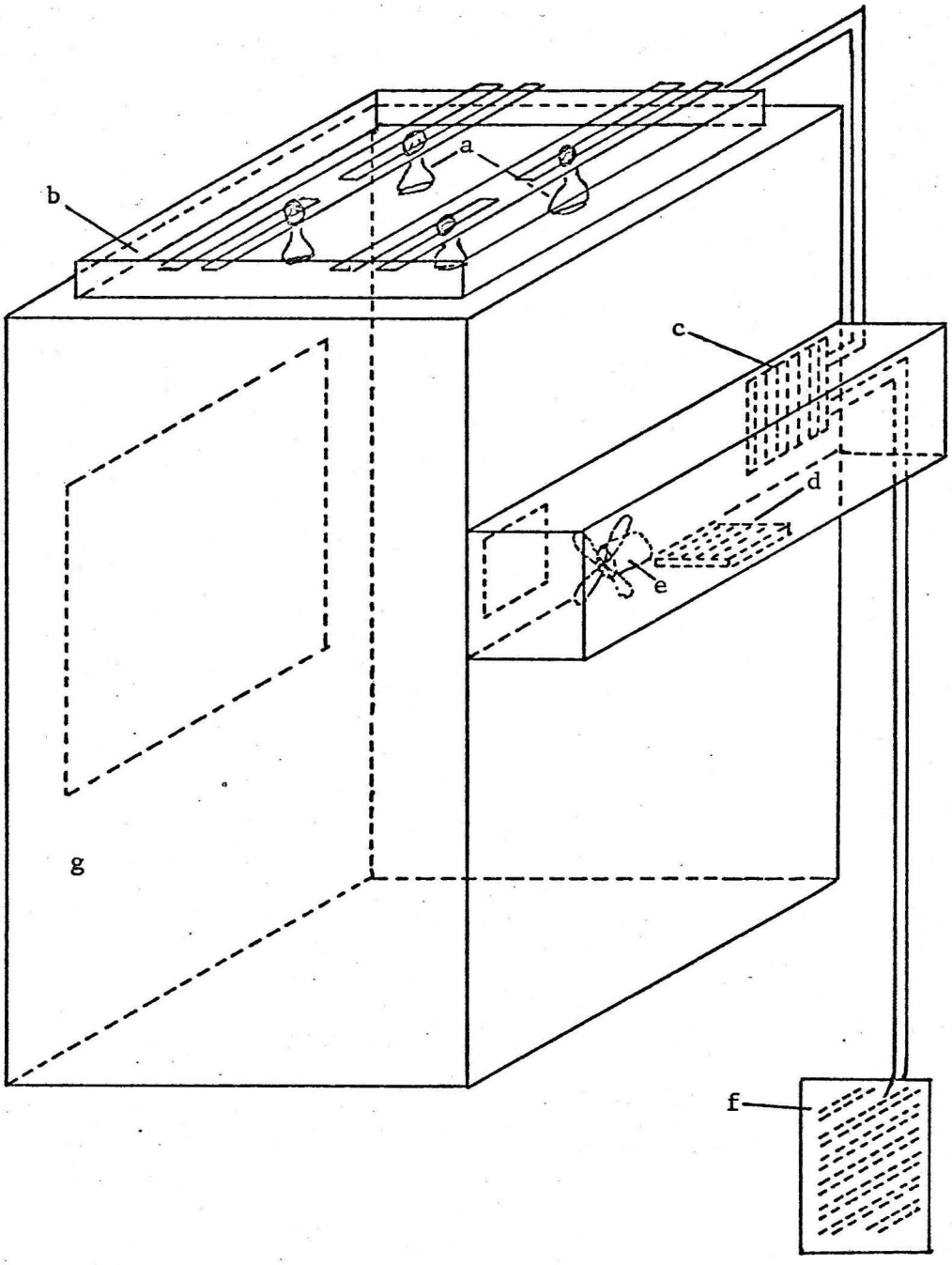
c. Verticle fin heat exchanger

d. Nichrome wire heater

e. Circulating fan

f. Refrigerated water bath

g. Plexiglass chamber


a
b
c
d
e
f
g

APPENDIX B

FIGURE 2. DIAGRAM OF THE TEMPERATURE CONTROLLED LEAF CHAMBER.

a. Plexiglass leaf chamber

b. Removable end plate

c. Slot through which leaf was inserted

d. air duct

e. Location of circulating fan and small heat exchanger

f. Water jacket

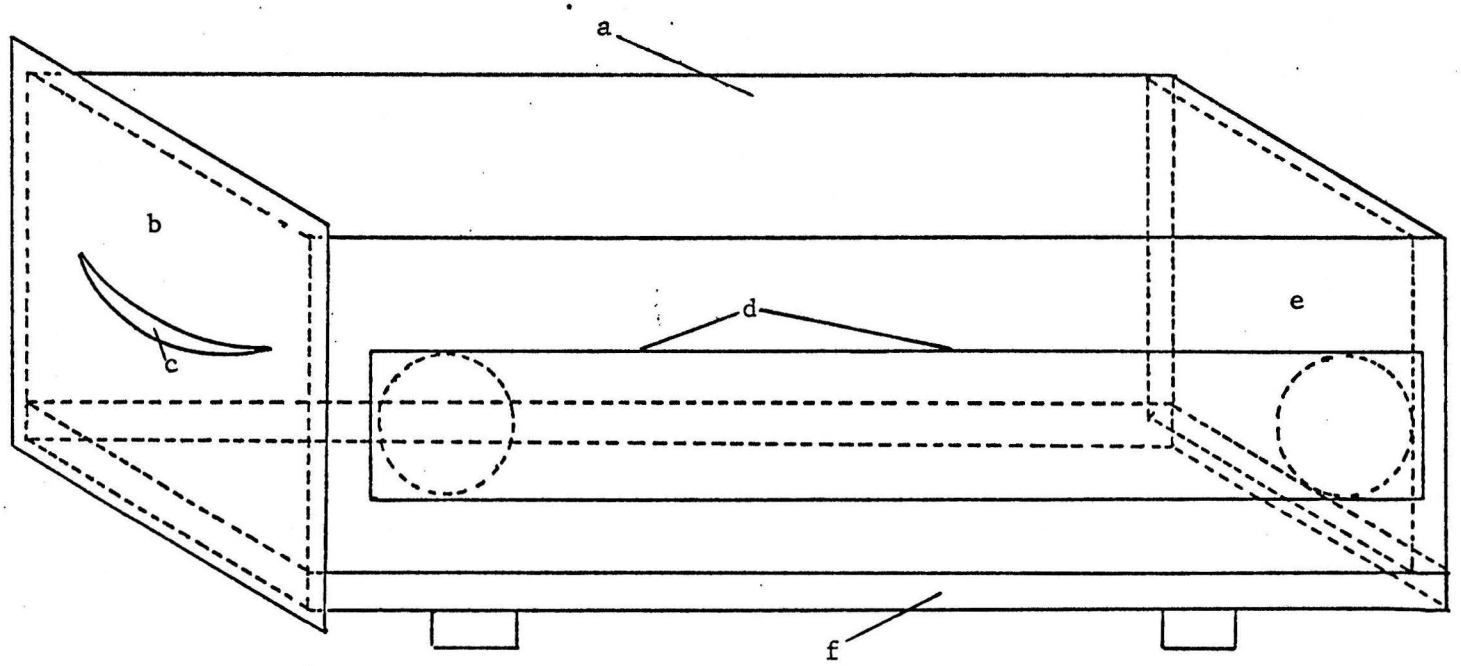
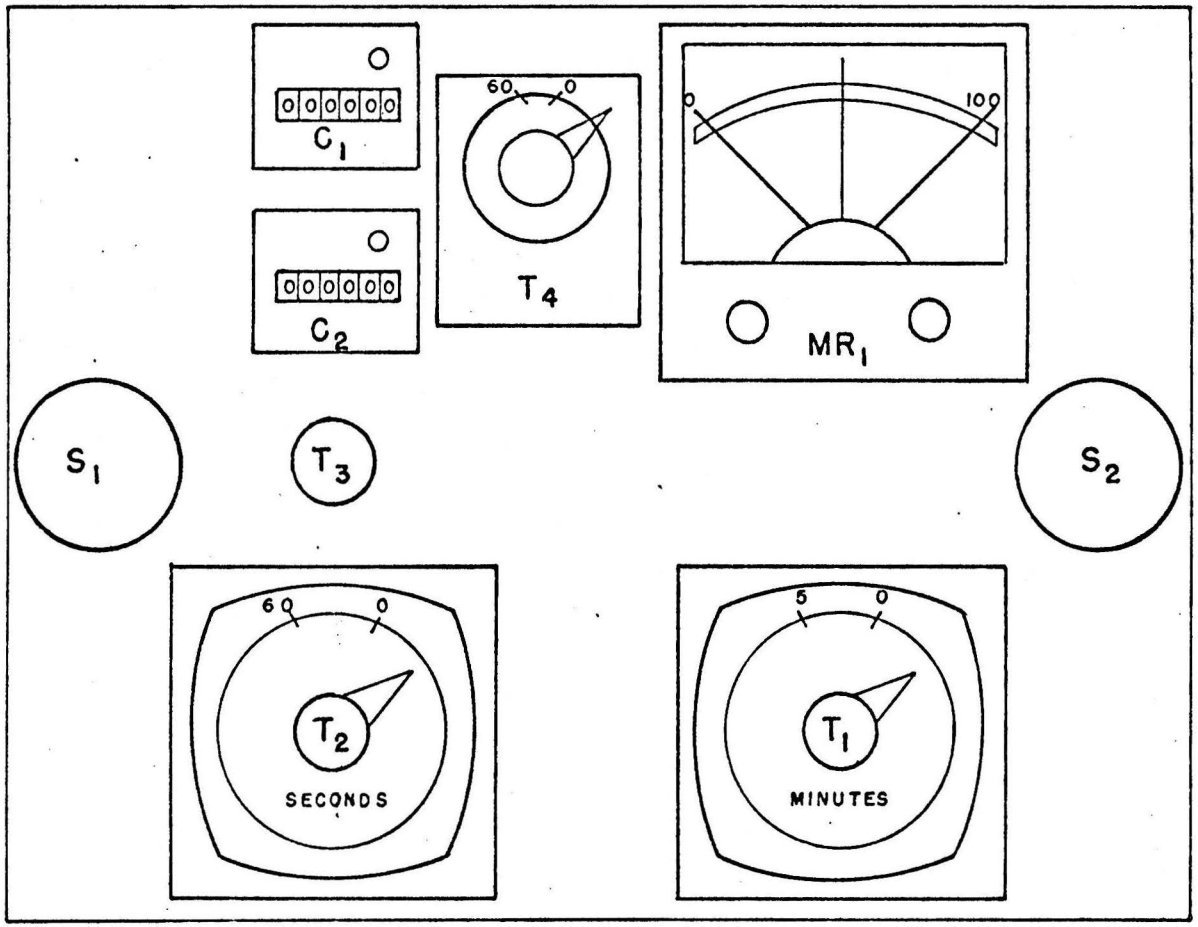

a
b
c
d
e
f

FIGURE 3. DIAGRAM OF THE CO_2 INJECTION AND REMOVAL CONTROL PANEL

MR_1	Double set-point control meter
S_1	CO_2 injection solenoid
S_2	CO_2 removal solenoid
C_1	CO_2 injection pulse counter
C_2	CO_2 removal pulse counter
T_1	CO_2 injection delay timer
T_2	CO_2 injection duration timer
T_3	30 second delay relay
T_4	CO_2 removal duration timer


C1
C2
60
0
T4
0
100
MR1
S1
T3
S2
60
0
T2
SECONDS
5
0
T1
MINUTES

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